DOCUMENT RESUME

ED 086 482 SE 016 645

TITLE	Handbook of Techniques and Guides for the Study of the San Francisco Bay-Delta-Estuary Complex, Part 1. Monitoring Techniques for the Measurement of Physico-Chemical and Biological Parameters.
INSTITUTION	Alameda County School Dept., Hayward, Calif.; Contra Costa County Dept. of Education, Pleasant Hill, Calif.
PUB DATE	Feb 71
NOTE	120p.
EDRS PRICE	MF-$0.65 HC-$6.58
DESCRIPTORS	Biological Influences; Ecological Factors; Environmental Criteria; *Environmental Education; Environmental Research; *Guides; *Instructional Materials; *Marine Biology; Natural Sciences; Quality Control; Resource Materials; Water Pollution Control
IDENTIFIERS	*California; Project MER; San Francisco Bay

ABSTRACT

Project MER (Marine Ecology Research) is aimed at improving environmental education in the San Francisco Bay Area schools. As part of meeting this goal, it is hoped that students and teachers can see the results of their efforts being put to practical use. This guide is the first of a series produced to help the students and teachers gather data concerning the San Francisco Bay-Delta-Estuary Complex and to organize these data in a form that could be a contribution to the literature of science and serve as the groundwork upon which knowledgeable decisions about the environment could be based. Presented in this guide are techniques and procedures for measuring and evaluating the ecology of aquatic environment of the Bay. Chapter I deals with how physical and chemical factors affect the distribution of aquatic life. General information on the effect of a particular factor precedes a technical presentation on how to measure or evaluate that factor. The second chapter discusses techniques for studying the plankton population and the third discusses techniques for studying bacterial populations. Field data sheets for recording data are included in the appendices. Related documents are SE 016 646--SE 016 650. (JP)

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PROJECT M E R

Marine Ecology Research

HANDBOOK OF TECHNIQUES
and
GUIDES FOR THE STUDY
of the
SAN FRANCISCO BAY-DELTA-ESTUARY COMPLEX

MONITORING TECHNIQUES:

FOR THE MEASUREMENT OF

PHYSICO-CHEMICAL AND BIOLOGICAL PARAMETERS

PART I

Contra Costa County Department of Education
Floyd Marchus, Superintendent

Alameda County Schools Office
Rock LaFleche, Superintendent

February 1971

SE 016 645

FOREWORD

Project MER is an outgrowth of a meeting of Contra Costa and Alameda County teachers and students who gathered a year ago to discuss their ideas regarding the teaching of aquatic ecology. This meeting, which may be called the beginning of MER, came about as the result of the sensitivity of Dr. William Landis, coordinator for science and mathematics for Contra Costa County, to the desires of active teachers wishing to improve instruction in environmental education. The group offered encouragement and direction for the development of a marine sciences program. All agreed that an opportunity had presented itself wherein students could become involved in something relevant and constructive. Specifically, those attending believed that students and teachers could see the results of their joint efforts in gathering data concerning the San Francisco Bay - Delta - Estuary Complex organized in such a form that it could be a contribution to the literature of science and serve as the ground work upon which knowledgeable decisions concerning the actions of governmental, industrial, and individual sectors of our society could be based.

At first, the problems of financial support, availability of shoreline access, building facilities for a marine laboratory, suitable research guides and supplemental materials seemed insurmountable. The first breakthrough appeared when the United States Navy granted a five-year lease on the use of land and buildings at Point Molate.

Shortly thereafter, with the support of the Associated Sportsmen, the Contra Costa County Board of Supervisors allocated funds for a six-month feasibility study and the hiring of a project director. In July 1970, they extended their support through June 1971. The Contra Costa County Department of Education has also contributed approximately $20,000 in supplies and services in support of this project. Colgate-Palmolive donated laboratory equipment and supplies and the Bay Area Biologists Society (B.A.B.S.) supplied an aquatic field testing kit. Moreover, several member companies of the Pittsburg-Antioch-Nichols Industrial Complex pledged monies to finance days of attendance at the marine station for high schools within their boundaries. Contra Costa College has granted Project MER computer facilities for the storage and dissemination of data to all participants and public agencies charged with conservation responsibilities.

All these donations paved the way for the operation of the program. The remaining funds necessary to implement the program were received in November 1970, by way of a $14,991.50 grant from the Rosenberg Foundation, enabling the training of students to begin in February 1971.

During the summer and fall of 1970, several teachers spent considerable time developing the *HANDBOOK OF TECHNIQUES AND GUIDE FOR THE STUDY OF THE SAN FRANCISCO BAY - DELTA - ESTUARY - COMPLEX*. It is difficult to credit all who were involved, but special mention must be given to Bob Davis, Jane Helrich, Ken Miller, Jim Shettler, Ed Springer, Neil Evans, Marcia Sakanashi, and Fred Tarp, without whose contributions the handbook would not have been possible. We were fortunate during the writing period to have the active support of consultants from California Fish and Game, the Federal Water Quality Agency, San Francisco State Water QUality Control Board, the Contra

Foreword - (Continued)

Costa County Water Agency, and the U.S. Department of Agriculture Soil Conservation Service. Particular appreciation is expressed to Arthur Noble of the Federal Water Quality Agency for his help in editing the *TENTATIVE KEY TO THE VERTEBRATES AND INVERTEBRATES OF THE SAN FRANCISCO BAY - DELTA - ESTUARY - COMPLEX.*

The importance of Project MER is best illustrated by the unstinting advice, cooperation and guidance of so many people and agencies. The entire clerical and professional staff of the Contra Costa County Department of Education has continually assisted in the development of MER. The cooperation of Alameda County Superintendent of Schools Office, by printing the Handbook in their publications facility, has made it possible for all the students involved to receive the necessary copies for their monitoring efforts.

We welcome your participation in this major step toward the solution of some of our ecological problems.

Director
Project MER

TO STUDENTS

"More than a century ago, when the Golden Gate was the entrance to El Dorado, San Francisco Bay became a world-wide symbol of opportunity and high promise. Now in a time of environmental crisis, it may once again be the destiny of this Bay to play that historic role."

Harold Gilliam, Between the Devil and the Deep Blue Bay

In order that your efforts as participants in Project MER be directed toward solving previously unanswered ecological questions concerning the San Francisco Bay - Delta - Estuary Complex, Dr. Fred Tarp, a leading authority on local Bay - Delta ecology has prepared the basic scientific research design for MER, which when implemented will serve to uncover important data as yet unavailable about this region.

As a student you will receive orientation to aquatic ecology from your instructor. When you visit the POINT MOLATE MARINE LABORATORY, you will have an opportunity to develop the field ecology skills necessary to carry on your research. With the skills mastered and the resource materials you will receive, you will be able to perform the necessary research required for the Monitoring Program of Project MER.

At specified times based on tides and seasonal flows you will be asked to monitor a FIELD STATION gathering data necessary for a better understanding of the bay complex. Your desire to contribute to this effort will enable the residents of this region to live in a better environment.

Under the Pilot Program beginning February 1971, 120 classes will be trained at the POINT MOLATE MARINE LABORATORY in the skills of aquatic ecology research. You are selected as one of these students and are fortunate to have this opportunity as we are to have you. The product of your efforts will be of extreme importance to the community. May this be a valuable experience for all.

Sincerely,

George J. Castellani

George J. Castellani
Director, Project MER

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M E R

MONITORING PROGRAM

CHAPTER I

TECHNIQUES FOR MEASURING PHYSICAL AND CHEMICAL FACTORS

in the

MARINE AND FRESHWATERS

of the

SAN FRANCISCO BAY - DELTA - ESTUARY COMPLEX

MER

INTRODUCTION TO THE SIGNIFICANCE OF PHYSICAL AND CHEMICAL FACTORS ON THE DISTRIBUTION OF AQUATIC LIFE

The range of all organisms is restricted by their environment. Amphibians such as frogs must sooner or later return to fresh water to lay their eggs; redwoods are restricted to regions of particular rainfall and temperature. To the neophyte, the many variations in "water" are easily overlooked. Yet, it is the many variations in the composition of natural waters which are important factors enabling different life forms to exist in one specific area and not in others. While many organisms can tolerate wide ranges in temperature and salinity (dissolved salts), others are highly specific in their requirements for life and can only exist when these conditions are present. Often species will migrate large distances following waters of specific temperatures. Other times, organisms will apparently disappear when water conditions change and reappear when the original conditions return.

ne' o phyte = novice, one not fully trained

sa lin' ity = saltiness of water, the more dissolved salt, the higher the salinity

It is important, then, when studying the many organisms which are found in the bay waters to study the physico-chemical conditions of the waters. It is possible from such studies to learn which factors limit the range of species and help us to understand their requirements. From a practical standpoint, such knowledge is invaluable to man. His presence, particularly in large numbers, usually results in "contamination" of natural waters. If there is sufficient knowledge of the requirements of the organisms which naturally inhabit these waters, man will be in a better position to predict the effects his actions will have on these organisms and, hopefully, modify his behaviors to prevent destruction of the environment.

physico-chemical = see listing below

range = the area where the organisms naturally occur

As part of this research program, selected physico-chemical factors will be monitored along the bay-delta-estuary complex. By amassing data on the physico-chemical conditions of the waters along with records of animal and plant populations, we will be able to supply the necessary information to make such predictions.

While there are many physical and chemical factors which can be measured and are important, the following are considered most critical and should receive primary consideration. These are:

CHEMICAL

Salinity	-	Total dissolved salts
Chloride ion	-	The major ion in salt water which has traditionally been used as an index of salinity.

CHEMICAL (Continued)

Phosphates	-	These chemicals act as fertilizers and are important in stimulating algal growth. In areas of high agricultural run-off, phosphate levels must be considered.
Dissolved O_2	-	As on land, oxygen is necessary for most animal forms. Different species require different levels of O_2.
Dissolved CO_2	-	Plant growth, as the result of photosynthesis, is directly related to the amount of dissolved CO_2.
pH	-	Degree of acidity or alkalinity of waters.

PHYSICAL

Current flow	-	Many organisms are limited by the rate of water flow.
Turbidity	-	"Cloudiness" of the waters caused largely by suspended silt or algal growth. Algal growth is directly related to the availability of light and nutrients.
Density	-	The density of water generally is related to the amount of dissolved salts found in the water. The greater the "density" the more "buoyant" the water is.
Substrate	-	This term generally refers to the land under a body of water. Often it serves as a storehouse of minerals, a home for many "burrowing" forms. The type of substrate is important in determining the kinds of organisms which populate a habitat.

photo syn' thesis = the complex process whereby green plants utilize water and CO_2 to produce food and O_2 in the presence of radiant energy (light)

silt = small soil particles

algal = refers to the algae, simple aquatic green plants

density = its relative heaviness

buoyant = having the ability to float

It would be convenient, though far less interesting, to the ecologist studying the waters if the physico-chemical properties of water were constant. Unfortunately for them, rivers flow, temperatures change, etc. Organisms have evolved to survive such natural fluctuations and often depend on

them for survival. In the laboratory, it is relatively easy to hold all the above variables but one constant and then measure the effect of this experimental variable on a specific organism. This has often been done. However, when one must study an actual river or bay system, he is forced to contend with situations where most of the physical, chemical and biological variables are constantly changing.

At each of the field stations which you will monitor during the year, your data on the physico-chemical conditions of the water will be placed on a computer program along with the biological data. As the result of constant monitoring at each field station over a period of years, the first complete picture of the seasonal changes in the properties of the inshore waters can be determined. This data can serve as the important "base-line" against which future changes in the bay can be compared. *The validity of the "base-line" will depend upon the accuracy and the completeness of the data collected.* When you begin to monitor a field station keep this in mind.

M E R

Introduction to the Chemical Tests Employed in this Handbook

COLORIMETRIC AND TITRAMETRIC TESTS

Most of the tests used in this HANDBOOK will fall into one of two distinct catagories, COLORIMETRIC or TITRAMETRIC. Both categories depend upon color change as a means for determining the concentration of an unknown substance in the sample which is being analyzed. In the COLORIMETRIC TESTS, an exact volume of a water sample is treated by the addition of an exact amount of one or more reagents. As the result of this treatment, a color will develop in the water sample. The *intensity* of the color which develops is related to the concentration of the unknown substance in the water sample. In the TITRAMETRIC tests, a reagent is slowly added to an exact volume of a water sample which has been previously treated. At some point when the reagent is being added, there will be a distinct color change called the "end point." The *volume of the titrant* which must be added to bring about this color change is directly related to the concentration of the substance in the water sample being tested.

In COLORIMETRIC tests, it is important that intensity of the color which develops be carefully identified in order that the concentration of the unknown chemical can be properly derived. This may be accomplished with the use of a COLOR COMPARATOR or PHOTOMETER.

A COLOR COMPARATOR may be either a series of vials of colored solution or a card with color markings. These comparators are prepared by first treating samples of water containing known concentrations of the chemical being analyzed. Vials containing the colored solutions or color cards are prepared by matching them to the colors produced by these concentrations and labeling them. When the test is performed on an unknown sample, the color produced is *matched* to the color on the comparator and concentration of the unknown is read directly from the comparator.

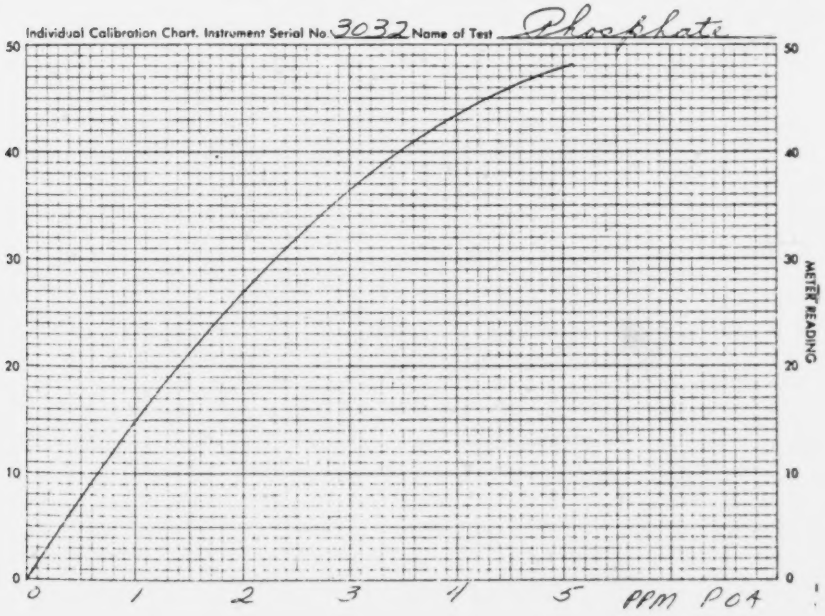
Accuracy using this technique depends upon the number of color samples on the Color Comparator, the degree of variation in color occuring when the concentrations of the unknown vary slightly, and the ability of the eye to discriminate between colors.

A PHOTOMETER is frequently employed to minimize the limitations of the Color Comparator. The photometer is an instrument in which a light beam passes through a filter which permits only specific wave lengths of light to pass through. The colored light is directed through a special glass test cell containing a treated water sample which then falls upon a photoelectric cell. The cell in turn produces a current proportional to the amount of light which reaches it and the current produced is read on a meter. The greater the intensity of the color, the greater is the amount of light striking the photo electric cell and the meter reading.

In order to determine the concentration of the unknown in the sample from the meter, a graph is prepared for each test, and the operator has only to locate the point where the meter value, which he reads, crosses the line on the graph.

For example:

> If in a test for phosphates, the meter value obtained were 0.30, the concentration of the phosphates using the graph below would be 0.20 ppm dissolved phosphates



It is important to understand how the graph was prepared. A series of carefully prepared phosphate solutions in concentrations ranging from 0 to 5 ppm were prepared and each one treated with the necessary chemicals and placed in the photometer. The meter reading for each concentration was marked on the graph and all the points connected. This graph then serves as the comparator against which readings from samples with unknown concentrations are compared. This procedure is followed in the preparation of each graph for all colorimetric tests.

The avoidance of many of the difficulties with Color Comparator methods is possible with the use of the photometer. However, it is important that the instrument be stable and carefully calibrated with the unknowns.

In both methods, it is *imperative* that all the glassware be scrupulously clean, the reagents used be of the highest purity and that concentrations be precise. The test vials and glassware should *not* be cleaned with soap since it is easy to leave a film containing traces of minerals on them which will result in erroneous readings. Wash the test cells and glassware *immediately* after use in tap water and rinse them in distilled water to prevent contamination.

If these precautions are taken, a photometer for use in colorimetric tests will yield accurate, replicable results with very little practice.

In TITRAMETRIC tests, as in colorimetric tests, all precautions mentioned concerning the preparation of reagents and cleanliness of glassware must be observed if accuracy is to be expected. In the titrametric tests in this HANDBOOK, a sample of water is treated chemically and a carefully measured sample (aliquot) is placed in a flask, beaker, or special container. Using a volumetric measuring instrument such as a burette, pipette, syringe with calibrations or dispensers which will release uniform drops, a reagent called the *titrant* is slowly added to the measured aliquot. At a certain point a distinct color change occurs. Since the strength and volume of the titrant and the volume of the aliquot are known, the strength (concentration) of the unknown in the aliquot can be easily calculated arithmetically using the proper chemical formula.

The accuracy of titrametric tests depends upon several factors. Obviously, the strengths and volumes of solutions used should be carefully measured. The quality of the measuring instruments is of utmost importance. In addition, the greater the amount of the sample employed in the test, the lower the percent of potential error. Why is this? For these reasons, each test has a basic acceptable error which is arrived at by considering all these factors. It is up to the researcher to decide how much error in each technique exists and if it is acceptable for his purposes.

During the preliminary stages of the Monitoring Program of Project MER, many methods for testing are being attempted and evaluated. Based on your experience, some of those tests now included may be modified, withdrawn and new ones included in future editions of this HANDBOOK.

DEVELOPING ACCURACY

After your training at the Point Molate Marine Laboratory, you will have an opportunity to practice various methods at your school until you become proficient at them. A good method to develop proficiency is to run a series of similar samples through the different procedures until you are able to obtain replicable results. With care, this should be quickly accomplished.

MONITORING A FIELD STATION

It is standard practice for all chemists to run duplicate tests for all samples. For example, when you collect a sample of surface water for analysis at a Field Station, you should make certain the enough water is collected to enable you to test it twice. If you do this, you will easily be able to determine if your technique is acceptable and the "technician error" is kept at a minimum.

MER

Tests for Dissolved Gases in Water

Prepared by Neil Evans

BACKGROUND:

The presence of adequate levels of dissolved gases, such as oxygen and carbon dioxide in water, is essential for the survival of most forms of aquatic life. Polluted water, in which dissolved oxygen and carbon dioxide may be almost non-existent, often contains only a few types of bacteria known as anaerobic bacteria. These organisms can survive in such a limiting environment because they do not use oxygen in obtaining energy from their food.

an aer o' bic

In the marine environment, the volumes of dissolved gases, in particular oxygen and carbon dioxide, vary considerably. Generally, increases in the salinity or temperature of waters reduces their capacity to hold gases in solution. Even under optimum conditions, gases are not very soluble in water. Along beaches where the surf breaks, or along streams where the water tumbles over rocks, considerable quantities of air are dissolved in the waters. In quieter waters, such as lakes, estuaries or tidepools, algae and other aquatic plants contribute oxygen as a by-product of photosynthesis. Carbon dioxide, a required "raw material" in the photosynthetic process is often supplied by animals and aerobic bacteria.

op' ti mum = ideal, the best

aer o' bic = require oxygen for respiration

The levels of dissolved oxygen and carbon dioxide in aquatic environments such as the Bay-Delta-Estuary Complex, can be affected in several ways. When raw or partially treated sewage enters the water, decomposition bacteria flourish. These aerobic bacteria remove large amounts of oxygen from the water. Sewage discharges also act as fertilizer for algae. Rapid growth and oxygen production occurs during the day. At night, however, more dissolved oxygen is consumed by animals, bacteria and algae and "die-offs" occur. The dead organisms add more organic material to the water further stimulating bacterial growth. Certain types of decompositon bacteria produce hydrogen sulfide, an obnoxious gas which is poisonous to most life forms. As the waters become saturated with hydrogen sulfide (H_2S) a "biological desert" appears.

Less obvious, but also important, are problems which arise when waters from agricultural lands return to the streams. In the eastern part of Contra Costa County, fertilizers from the farms enter the slow moving canals of the delta. This runoff, rich in phosphates $(PO_4)^{=}$ and nitrates $(NO_3)^{-}$, supplies the algae with some of the requirements of growth in much the same way that fertilizers increase growth of land plants. However, when night comes, the fish and plants require oxygen for survival. It takes little time for the plants and animals to

consume the dissolved oxygen. As the result, "oxygen depletion" rapidly occurs and organisms begin to die. Bacterial decay results and gases produced in the process can further limit the amount of oxygen and carbon dioxide the waters can hold. Such a process can lead to rapid die-offs and the lowering of general water quality.

As part of the MER Monitoring Program, tests for a series of gases will be undertaken. From this data you will be able to detect changes in the gas concentrations and correlate these with changes in animal populations, an important step in understanding the ecology of an aquatic system.

M E R

Carbon Dioxide Determination

Prepared by Bob Davis

BACKGROUND:

Carbon dioxide is very soluble in water and can be expected to be found in highly variable amounts. Water obtains its source of CO_2 from animal respiration, decay, and soil or underground sources. E.P. Odum in Fundamentals of Ecology, states: ...the "minimum" is less likely to be of importance than is the case with oxygen. Furthermore, unlike oxygen, carbon dioxide enters into chemical combination with water to form H_2CO_3, which in turn reacts with available limestones to form carbonates $(CO_3)^{=}$ and bicarbonates $(HCO_3)^{-}$ These compounds not only provide a source of nutrients but also act as buffers, helping to keep the hydrogen ion concentration of the environment nearer neutral. He further indicates that the behavior of CO_2 in water is different from oxygen and its ecology is not yet well known, and it is therefore difficult to make general statements as to its role as a limiting factor.

nu' tri ents = Material taken in by living organisms which serve to sustain and promote growth within the organism.

M E R

Carbon Dioxide
Titrametric Determination (Delta)

INTRODUCTION:

Dissolved carbon dioxide concentration in local waters may vary for many reasons. During the day large amounts may be utilized by plants during photosynthesis. In the evening the combined activity of plants and animals can result in an increase in the carbon dioxide level.

This test is based on the addition of standard sodium carbonate to a sample of eater in the presence of the indicator, phenolphthalein. The carbon dioxide reacts with the sodium carbonate to form sodium bicarbonate which is colorless to phenolphthalein. As soon as an excess of sodium carbonate is present, a red color appears and this is taken as the endpoint of the titration.

MATERIALS:

Water Samples: Surface, 1 meter and bottom
Plastic Sample Tube No. 8 (Delta)
Reagent No. 25 (Thiosulfate)
Reagent No. 3 (Phenolphthalein)
Reagent No. 24 (Standard Sodium Carbonate)

METHOD:

1. Rinse the No. 8 Plastic Sample Tube with the water being tested and then fill exactly to the mark. (The curved surface or meniscus should be exactly even with the mark). If chlorine or bromine is present, add 2 drops of Reagent No. 25 (Thiosulfate) and mix gently.

2. Add two drops of Reagent No. 3 (Phenolphthalein) If the sample turns red, no free carbon dioxide is present.

3. If the sample remains colorless, add Reagent No. 24 (Standard Sodium Carbonate) drop by drop (keeping count of drops), swirling the sample gently, until a definite pink color develops throughout the sample which remains permanent for 30 seconds. This is the endpoint.

4. Each drop of Standard Sodium Carbonate with a 25 ml sample is equal to 5 parts per million (ppm) of free carbon dioxide (CO_2). Free mineral acids, if

METHOD:

4. (Contunued)
present, will be measured by this test. Iron and aluminum salts will interfere. Some loss of carbon dioxide may occur, so the addition of Standard Sodium Carbonate should be completed as soon as possible. Mixing should be as gentle as possible, to avoid loss of carbon dioxide. RECORD the number of drops on the Data Sheet.

5. Repeat the above test on the remaining samples and RECORD on the Data Sheet.

6. Calculations: Since each drop of Standard Sodium Carbonate with a 25 ml sample equals 5 ppm of free carbon dioxide, multiply the number of drops used in each sample to calculate the amount of CO_2 present in ppm. RECORD on the Field Data Sheet.

M E R

La Motte Carbon Dioxide Test

Prepared by Bob Davis

MEASUREMENT:

Measurements of carbon dioxide concentration will be made by use of the La Motte Chemical Test Kit for Carbon Dioxide.

MATERIALS TO TAKE TO SAMPLING STATION:

The entire test kit: Carbon Dioxide Reagent
Phenolphthalein Indicator
Sample Bottle - titration tube
Micro burette

AT THE SAMPLING STATION:

1. Obtain a sample with a minimum of contact with the air (avoid splashing, etc.) Fill the titration tube to the mark indicated with the water sample.

2. Add 2 drops of Phenolphthalein reagent. If the sample turns red, no free carbon dioxide is present. Undoubtedly this will not happen and the solution will remain colorless.

3. Titrate with the Carbon Dioxide Reagent by using the micro-burette, until a faint but permanent pink color is produced and persists for at least 30 seconds. The sample should be gently agitated during this titration procedure.

   To use micro-burette: Fit micro burette into cap of carbon dioxide reagent bottle. Invert and fill micro-burette by withdrawing the plunger. Fill until the burette reads zero. Turn the bottle right side up and remove the burette. Place the burette in the larger hole of the titration tube cap. Depress the plunger to expel the titration solution. A slight rotating or twisting motion permits the plunger to move smoothly. Agitate the titration tube so that each addition of the titrating reagent is thoroughly mixed with the sample.

   After you have a permanent pink color for at least 30 seconds, make a reading of your burette. Each major division on the burette equals 2.5 ppm carbon dioxide and each minor division is 0.5 ppm. RECORD your results on the data sheet.

MER

Oxygen

Prepared by Bob Davis

BACKGROUND

The primary life limiting gas in water is oxygen (O_2). It is interesting to realize that while there may be as much as 210 ml of oxygen in a liter of air under the most favorable conditions, the amount of oxygen in water will not exceed 10 ml per liter. The amount of oxygen which the water can dissolve is limited by such factors as temperature and salinity. Generally, the higher the salinity and temperature, the lower the amount of O_2 which the water is capable of holding.

MEASURING THE DISSOLVED OXYGEN:

There are two basic methods for measuring the amount of dissolved oxygen found in water:

A. Titration methods: This procedure involves a chemical reaction in which the dissolved oxygen is combined with other chemicals. The investigator, using a series of chemicals, measures the volume of the newly formed chemical. From this data it is possible to determine the amount of dissolved oxygen originally present in the sample.

B. Direct reading meter methods: Meters with special membranes have been designed which can be used to measure the amount of dissolved oxygen directly.

Since meters are not always available and it is difficult to know whether they are properly "calibrated," it is standard laboratory practice to utilize both methods to determine the O_2 content of water. When one is certain that the meter is "properly functioning" the direct reading meter is generally employed since it is quicker and simpler to use. For the purpose of this research, investigators must be competent with both methods. The meter may be used <u>only</u> when one is certain that the results are valid.

cal' i brate = to make certain that the values indicated on the meter are accurate.

Oxygen Determination
Winkler Titration Method (Rideal-Stewart Modified)

Prepared by Bob Davis

INTRODUCTION:

The Rideal-Stewart modified Winkler method for determining oxygen is ideally suited for waters which may contain quantities of iron such as is suspected in our Bay. Other variations of the method exist, but this method is the most commonly employed under these conditions.

Unlike water in a fish tank where a sample taken at any portion would be similar in its properties to a sample of water taken from another portion, waters in a bay or a big pond vary considerably from area to area. Perhaps you have had the opportunity to dive into a lake in the spring and have noticed that as you reach a certain depth, you will contact a layer of very cold water. This is called the thermocline. Such layering of waters due to temperature and salt concentration (this layer is called the halocline) are common. Variations of the other physical and chemical factors are also common. It is therefore necessary, when studying the properties of a water system, to analyze samples at different depths at the same station. Often, populations of different animals and plants are restricted to different depths because of these conditions. As you carry out tests to determine the various properties of water taken at a given station, it is important that you include information on the properties of the waters at the station at various depths.

ther' mo cline

hal' o cline

METHOD: For Collecting Water Sample for O_2 Analysis

A. Since the water is to be analyzed for dissolved oxygen, sample must be collected in such a fashion that air is not bubbled through the sample. Oceanographers and limnologists (those who study fresh water) have developed special equipment for gathering such samples. In all samples the water can be taken from the sampler in a fashion which prevents or minimizes mixing.

B. The water sample of at least 500 ml should be carefully run into a glass stopper bottle of at least 250 ml capacity by inserting a delivery tube in the bottom of the bottle. The water should be allowed to overflow until the sample bottle has had several volumes passed through it to flush out the atmospheric oxygen. When the bottle is filled, the glass stopper is inserted carefully to avoid the introduction of any air bubbles. A record of the water temperature in the collecting sample must be taken at this time for further use. RECORD temperature.

MATERIALS:

(Items marked with * should be in the field kit.)

* 1. Bottle for sample
* 2. Glass bead
3. 3 each 1 ml pipettes marked as follows:
   *a. H_2SO_4
   *b. $KMnO_4$
   *c. $MnSO_4$
   *d. $K_2C_2O_4$
* 4. Concentrated sulfuric acid (H_2SO_4)
* Potassium permanganate ($KMnO_4$)
* Manganous sulfate ($MnSO_4$)
* Potassium oxalate ($K_2C_2O_4$)
Sodium thiosulfate ($Na_2S_2O_3$)
Starch solution
Hydroxide sodium iodide (KOH-NaI)
Calcium carbonate ($CaCO_3$)
5. 2 each, 5 ml pipettes marked as follows:
   *a. KOH-NaI
   b. Starch
* 6. Apparatus for the collection of the water sample
* 7. Thermometer
8. Burette, 25 or 50 ml
9. Burette stand
10. 200 ml volumetric flask
11. 250 ml Erlenmeyer flask

METHOD: The first 7 steps should be completed as soon as possible, preferably in the field.

NOTE: When adding reagents, use the pipette which is clearly marked with the name of the reagent. The pipette should be inserted well into the bottle to prevent the reagent from spilling over. Gradually withdraw the pipette, making certain that all the reagent has been released below the neck of the bottle.

1. Carefully collect a water sample as previously described, RECORD the water temperature, then:
2. Remove the glass stopper from the water sample bottle and to the sample add:

   a. a glass bead to aid in mixing
   b. CHECK WITH YOUR INSTRUCTOR BEFORE CARRYING OUT THIS STEP
      0.6 ml of concentrated sulfuric acid (H_2SO_4)
      WARNING: Do NOT add an excess.
   c. 1.0 ml of potassium permanganate solution ($KMnO_4$)
3. Carefully replace the stopper, again being careful to avoid the introduction of air. SHAKE WELL.

METHOD:

3. (Continued) A pale violet to pink color should appear and persist. If it does not, add another ml of $KMnO_4$ solution and shake well. The color should now appear and persist for five minutes.

4. Add 1.0 ml of potassium oxalate ($K_2C_2O_4$) solution. Stopper, shake and let stand until the color disappears. Let stand in the dark for ≈ 2--10 minutes. This is called the Contact Period.

5. To the sample add:
   a. 2.0 ml manganous sulfate solution ($MnSO_4$)
   b. 3.0 ml hydroxide sodium iodide solution (HOH-Na-I)

6. Stopper and shake well. A yellow precipitate will form. Allow this to partially settle and shake again.

7. CALL YOUR INSTRUCTOR BEFORE CARRYING OUT THIS STEP:

   Add 2.0 ml of concentrated H_2SO_4 to the sample. stopper and shake. The precipitate should dissolve. If it does not, add another 0.5 ml of the acid, stopper and shake. (The yellowish color which remains represents the iodine which has replaced the dissolved oxygen.)

pre cip' i tate = a solid which appears in a solution and tends to settle to the bottom of the container

The preceding operations have resulted in releasing an amount of iodine equivalent to the amount of oxygen originally present in the bottle. The solution is relatively stable and can be readily transported. However, it is important to keep the bottles cool and out of the sunlight until you reach the laboratory. Complete the following steps within 36 hours.

The following part of the Winkler method involves measuring the amount of iodine present. From this information you will calculate the amount of oxygen which was originally in the sample.

8. Measure out 200 ml of the sample using a volumetric flask. Pour this into a clean 250 ml erlenmeyer flask. If boiled, distilled water is available, add a ml or so to the volumetric flask, carefully swirl the water and pour the liquid into the erlenmeyer flask with the solution.

   It is essential that the water used be cool (room) temperature.) If the water is warm, the iodine may be released into the atmosphere.

vol u met' ric flask = a special piece of glassware designed to measure a specific volume accurately

METHOD: (Continued)

9. Fill a burette with the sodium thiosulfate ($Na_2S_2O_3$) solution. Run a small volume through the burette into a waste container to free the burette of air bubbles. RECORD the level of sodium thiosulfate in the burette on the Data Sheet. Begin to titrate the sodium thiosulfate into the sample flask. This may be done fairly rapidly, but be careful to note the disappearance of the iodine and stop when the solution is a pale yellow. It takes practice to avoid adding too much thiosulfate.

bu rette'

ti' trate = the act of releasing a solution from a burette

10. Add 2 ml of the starch solution. The sample will turn blue. (If it does not, you have added too much thiosulfate and must begin again.)

11. Continue titrating slowly until the sample becomes clear. The clearness should persist when the solution is agitated. Be careful to make certain that you add the minimum amount of thiosulfate which will result in the clear solution. If you have any questions, ask your instructor. RECORD the level of thiosulfate in the burette. Calculate the volume of thiosulfate used. RECORD this value.

12. CALCULATIONS:

The amount of oxygen in parts per million (ppm) may now be calculated with the following formula:

$$\text{ppm dissolved } O_2 = \text{Vol. of thiosulfate} \times \text{Normality of thiosulfate solution} \times 40$$

13. It is standard practice to obtain two identical samples from <u>each</u> depth. The ppm D.O. at each depth should theoretically be identical. In practice, small variations will occur. If the ppm D.O. values obtained from the matched samples are close, one is reasonably certain that his results are accurate.

Average the paired results and RECORD for <u>each</u> set of samples.

METHOD: (Continued)

14. Derivation of the formula for calculation of dissolved O_2 in ppm.

$$\text{ppm D.O.} = \frac{\text{ml thiosulfate X Normality (thio.) X mew}}{\text{sample size}}$$

Milliequivalent weight (mew) of $O_2 = 0.008 \times 10^6$

Therefore:

$$\text{ppm D.O.} = \frac{\text{ml thiosulfate X Normality (thio.) X } 8{,}000}{205} \approx$$

ml thiosulfate X Normality (thio) X 40

M E R

HACH TITRATION METHOD FOR DISSOLVED OXYGEN DETERMINATION (LAB)

Prepared by Ed Springer

INTRODUCTION:

The Hach Method for Dissolved Oxygen Determination is a variation of the Winkler Technique. The investigator should read the general introduction to the Rideal - Stewart Modification of the traditional Winkler Technique for Dissolved Oxygen for background and information concerning collection of water samples. A major advantage of this technique is that it involves the use of PAO (Phenylarsene Oxide) instead of a Sodium Thiosulfate Solution. This chemical is more stable than the traditional thiosulfate and is packaged in quantities ready for use in a single test using a standard 300 ml size BOD sample bottle.

MATERIALS:

Manganous sulfate
Alkaline Iodide-azide
Sulfamic acid
Starch indicator solution
PAO solution
Titration apparatus
...Supplied in dry, premeasured packages called "pillows."

PRECAUTIONS:

In this method,as with the previous methods, the investigator carefully notes all physical factors, i.e., tidal conditions, wind, etc., on the data sheet since they can be responsible for temporary or local variations in dissolved oxygen in samples. Unusual care in obtaining samples in shallow waters where there is tidal disturbance must be taken, or unreliable readings will result.

METHOD:

1. Fill a standard 300 ml BOD bottle or other suitable sampling bottle with the water to be tested according to the procedures previously outlined in the Rideal - Stewart Method. Again, be sure that there are no air bubbles present. The use of a Van Dorn or other water collecting apparatus is advised.

2. Add the contents of one manganous sulfate powder pillow (or 2 ml of manganous sulfate solution) to the water sample bottle. If a solution is used, insert the pipette well into the water sample so the solution being added will not flow out of the bottle without mixing with the water sample.

METHOD: (Continued)

3. Add the contents of one Alkaline Iodide - Azide Powder Pillow (or 2 ml of Alkaline Iodide-Sodium Solution, using a pipette as described in 2) to the BOD bottle.

4. a. Restopper the bottle in a manner to exclude all air bubbles. This can be easily accomplished by tipping the BOD bottle slightly and then inserting the stopper at an angle with a quick downward thrust.

   b. Shake the bottle very well to mix the chemicals and the floc that forms.

   c. Allow the floc to settle about half way down the bottle. (The time for settling varies with the sample.)

5. a. Clip open one Sulfamic Acid Powder Pillow.

   b. Remove the stopper and add the reagent to the sample bottle.

      (2 ml of concentrated Sulfuric Acid may be used in place of Sulfamic Acid. CAUTION: Check with your instructor before adding concentrated Sulfuric Acid.)

   c. Restopper carefully, and shake to mix. The floc will dissolve and a yellow color will develop if oxygen is present.

      [At this point all biological action has been stopped by the three reagents and the oxygen is "fixed." The above steps must be carried out in the field. The following steps can best be completed back at the laboratory.]

6. a. Fill a 200 ml volumetric flask to the scribed line or measure 200 ml of the solution of the BOD bottle in a graduated cylinder.

   b. Pour this measured volume into a 300 ml erlenmeyer flask.

7. Using the Standard PAO Solution, titrate into the 200 ml water sample until it is pale yellow.

8. Add 2 ml of Starch Indicator Solution. A blue color will be formed. If the black does <u>not</u> appear, you have added too much PAO. Begin again.

METHOD: (Continued)

9. Continue the titration until the blue color just disappears. (Check with your instructor.)

10. The ppm Dissolved Oxygen is equal to the number of mls of PAO used. RECORD on your Data Sheet.

M E R

La Motte Titration Method for Dissolved Oxygen Determination

Prepared by Bob Davis

INTRODUCTION:

Use of the La Motte Dissolved Oxygen Set as an alternative measurement of oxygen. (The investigator should read the general introduction to the Rideal-Stewart modification of the traditional Winkler test for dissolved oxygen.)

MATERIALS:

La Motte Dissolved Oxygen Set
Materials to take to sampling station:

The entire test kit - Sampling bottle
Manganese Sulfate Solution
Potassium Iodide Solution
Sulfuric Acid
Sodium Thiosulfate Solution
Starch Solution
Pipette
Micro burette
Titration flask

METHOD:

At the Sampling Station:

1. The techniques for the collection of water using a water sampler should be followed for this method as is followed for the technique for water collection outlined in the Rideal-Stewart modification of the Winkler technique.

2. RECORD the temperature of the water and depth the sample was taken.

3. Place the hose from the water sampler into the bottom of the sample bottle. Let the water flow into the bottle overflowing at least 3 times the volume. Be careful to prevent any air bubbles from collecting on the side of the bottle. Place the cap on the bottle carefully and invert the bottle to make certain that no air is trapped inside.

4. To the sample add 8 drops of Manganese Sulfate solution and 8 drops of Alkaline-Potassium-Iodide Solution. Carefully cap the bottle, mix by gently inverting, then allow the precipitate to settle below the shoulder of the bottle.

METHOD: (Continued)

5. CALL YOUR INSTRUCTOR BEFORE PROCEEDING. Add one measure (0.5 ml) of Sulfuric Acid, cap the bottle and mix until the precipitate is completely dissolved.

LABORATORY TEST:

1. Fill the titration flask to the 50 ml line with the water sample.

2. Fill the Micro Burette with standard Sodium Thiosulfate Solution

   To use Micro Burette - Fit Micro Burette into cap of Sodium Thiosulfate Solution. Invert and fill micro burette by withdrawing the plunger. Fill until the burette reads zero. Turn the bottle right side up and remove the burette. Place the burette in the hole of the titration flask cap. Depress the plunger to expel the titration solution. A slight rotating or twisting motion permits the plunger to move smoothly. Agitate the titration flask so that each addition of the titrating reagent is thoroughly mixed with the sample.

3. Continue to titrate with the standard Sodium Thiosulfate Solution until the brown iodine color has nearly disappeared. Remove the micro burette and cap and add 8 drops of starch solution. The sample will turn blue. Now continue the titration until the blue color just disappears.

4. Each major division on the micro burette is equivalent to 0.2 ppm dissolved oxygen and each minor division 0.04 ppm. RECORD the values.

   Example of calculation - If 18 major and 2 minor divisions are needed to reach the titration end point, the calculations would be: (18 x 0.2) plus (2 x 0.04) or 3.68 ppm dissolved oxygen.

5. RECORD your results on the data sheet.

MER

Dissolved Oxygen Determination of Water Using the Delta Model 85 Oxygen Meter

Prepared by Ken Miller

INTRODUCTION:

The use of the oxygen meter provides a quick means to measure dissolved oxygen. As with the use of any meter, care must be taken to make certain that the meter is functioning properly and the readings are accurate. For this reason, one should run a parallel test for dissolved oxygen using a titration method on a duplicate sample of water, i.e., a Winkler test, prior to taking the meter out of the laboratory. If the results agree, you can feel certain that the meter is functioning properly and that the data you obtain is valid. The dissolved oxygen readings you will be gathering when monitoring a Field Station are one of the most important pieces of data you will gather, so make certain it is done carefully.

MATERIALS:

Delta Model 85 Oxygen Meter with agitator
Water Sampler
BOD bottle

METHOD:

ADJUSTMENT AND CALIBRATION OF THE METER

Your teacher has the instructions for adjusting and calibrating this instrument. DO NOT ATTEMPT TO DO SO YOURSELF.

DAILY PREPARATION OF THE METER FOR USE

If this instrument is being used for the first time today, have your teacher place the BOD Bottle Sample Agitator on the Probe and adjust it for you. If it has been used today, carefully remove it from the holder and carry it to the water sampling site.

To check the meter for accuracy before going into the field, fill a one liter container (or equivalent) 1/2 full with distilled water. Shake it well to cause O_2 to dissolve in the water. Following the FIELD PROCEDURE on the next page, determine the concentration of dissolved O_2. Using the same sample, follow one of the titration procedures in this manual to determine the dissolved O_2. When you have completed your calculations, the value obtained by titration should not differ by $\pm$ 5% the value obtained by the meter method.

METHOD: (Continued)

FIELD PROCEDURE:

1. Fill a BOD Bottle with water from the water sampler as per directions given in the section entitled the Rideal-Stewart Modified Winkler Test.
2. a. Place the probe with the agitator into the BOD Bottle.
   b. Turn on the agitator (connect wires to battery.)
   c. Turn the Function Selector Knob to READ TEMPERATURE.
   d. Wait 2 or 3 minutes (until the needle stops) and read the temperature in °C on the red temperature scale of meter. RECORD
3. Rotate Temperature Setting Control to the exact temperature you just recorded.
4. Turn the Function Selector Knob to READ OXYGEN.
5. a. Read the dissolved oxygen directly from the meter. RECORD
   b. RECORD number of the BOD bottle and save the sample if any other tests are to be performed.
6. Turn off the agitator (disconnect wires to battery) before removing the probe from the BOD bottle.
7. Repeat steps 1 through 6 for each water sample you are testing.

RETURN TO LABORATORY WITH BOD BOTTLES AND METER. Place meter back in its holder immediatly on return.

LABORATORY PROCEDURE:

8. If water is saline, determine salinity of each sample by one of the methods discussed in this handbook.
9. Reserve the sample if other tests are to be made.

CALCULATION OF OXYGEN DISSOLVED IN SALINE WATER

10. Use the Nonograph on the next page to obtain the corrected oxygen reading for the samples.
    a. Place a ruler edge on the values you obtained on the temperature and salinity scales. RECORD to the value where the ruler's edge crosses the oxygen solubility or middle scale. (Example: If the temperature of the sample equals 9° C and the salinity equals 25°/₀₀, then the oxygen solubility is 9.6 mg/L.)
    b. Place the ruler on the temperature scale and on the salinity scale at 0°/₀₀ (distilled water.) Example: If the temperature equals 9° C and the salinity equals 0°/₀₀, the oxygen solubility is 11.2 mg/L)

METHOD: (Continued)

c. Divide the value obtained in step 10.a by the value obtained in step 10.b and multiply this value by the reading on the meter obtained in step 5a.

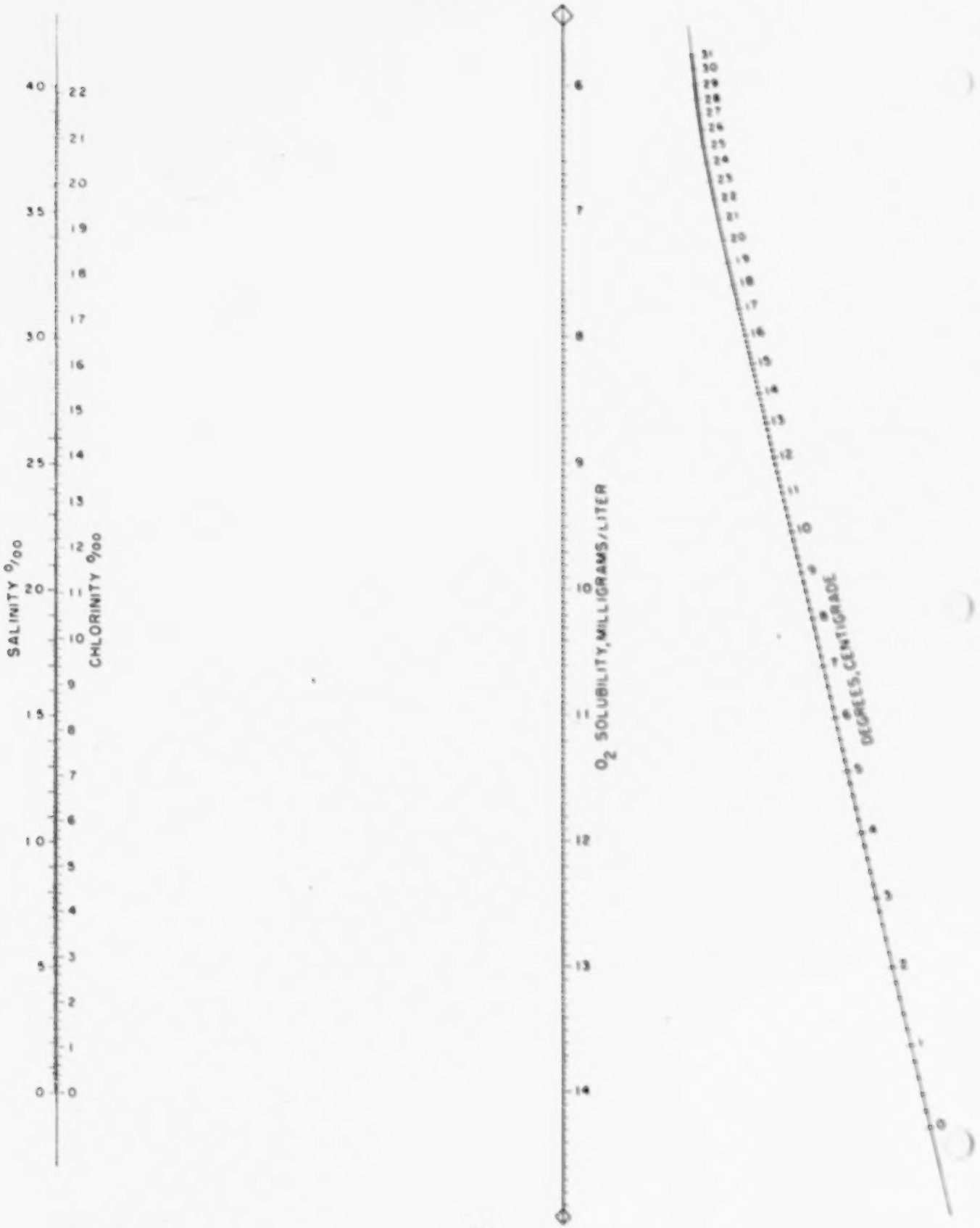
$$\frac{\text{Step 10.a}}{\text{Step 10.b}} \times \text{Step 5.a} = \text{mg/l dissolved oxygen in sample.}$$ RECORD

Example:

$$\frac{9.6 \text{ mg/l}}{11.2 \text{ mg/l}} \times 14 \text{ mg/l} = 11.9 \text{ mg/l}$$

Oxygen concentration is often measured in ppm. Since 1 ppm = 1 mg/l, conversion to ppm can be simply accomplished.

NONOGRAPH


SALINITY °/00
CHLORINITY °/00
O_2 SOLUBILITY, MILLIGRAMS/LITER
DEGREES, CENTIGRADE

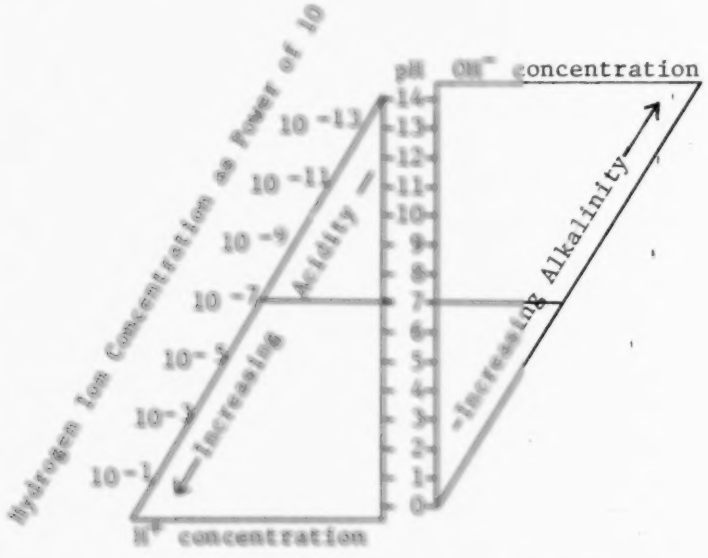
MER

Acidity and Alkalinity of Sea Water

Prepared by Bob Davis

BACKGROUND:

Sea water is normally alkaline. In a pure water, or in any solution that contains equal concentrations of hydrogen and hydroxyl ions, the solution is considered to be neutral. For example, pure distilled water disassociates into hydorgen and hydroxyl ions $(OH)^-$ of equal number. If the concentration of H^+ is in excel of $(OH)^-$, the solution is acid, and if less, it is considered alkaline. For expressing the hydrogen ion concentration a logarithmic scale is commonly used, and the neutral position is 7 on a 1 through 14 scale. The numbers 1 to 7 indicate the acid range, and the numbers above 7 to 14 represent the alkaline range. However, it must be kept in mind that by the use of a logarithmic scale, a unit change in pH corresponds to a tenfold change in the hydrogen ion and hydroxyl ion concentrations.



Both the external and internal components of organisms are subject to considerable change if there are variations of the acidity (or alkalinity) of their waters. The chemical processes that occur in living cells are carried on in solutions relatively near to neutrality. Special mechanisms are maintained in cells and in the body fluids to keep the hydrogen ion concentration constant. These mechanisms are called buffers. These buffer systems, such as one found in the blood of organisms, keep the fluids relatively constant and maintain an ionic equilibrium similar to that which exists

BACKGROUND: (Continued)

in pure water. However, these have no control over the external environment of the organisms, and this pH of the external environment plays a key role in the adaptability of the organism to its existing habitat.

It therefore becomes important to measure the external environment in terms of acidity and alkalinity, as the nature of the pH limits the kinds of organisms which can exist.

There are three techniques which can be easily employed to measure the pH of sea water. One is by use of special pH paper which is readily available, the second method employs the use of special pH meters, and the third, a titration method.

M E R

METER METHOD FOR DETERMINING pH

Prepared by Bob Davis

(Many acceptable meters are available. The following instructions are for the Beckman Portable pH Meter, Model 180.)

MATERIALS TO TAKE TO THE SAMPLING STATION:

Beckman pH meter.

Buffer vial with sponge which has been saturated with pH 7 buffer.

Distilled water in small flask.

Prior to taking meter from lab, fill the electrode with KCl and AgCl solution.

Kleenex type tissue.

FOLLOW THIS PROCEDURE PRIOR TO VISIT TO SAMPLING STATION:

1. Turn meter switch to "on" position.
2. Slide electrode sleeve (plastic sleeve on electrode) down below filling hole. Check KCl-AgCl level: refill if necessary.
3. Slide electrode sleeve back over filling hole.

AT THE SAMPLING STATION:

4. Slide electrode sleeve down.
5. Remove cap from electrode tip, and rinse electrode in distilled water. Dry with Kleenex type tissue.
6. Insert in buffer vial and press enough buffer from the sponge to make contact with the electrode tip.
7. Turn adjusting knob (marked ADJ) until meter reads pH 7.
8. Hold down CHECK button and set Memory Dial (lower left hand corner of instrument) to meter reading of 7. Release CHECK button.
9. Remove electrode from buffer solution, rinse in water, dry with tissue, and recap buffer vial.
10. Collect water sample.

AT THE SAMPLING STATION: (Continued)

11. Place electrode in water sample and read pH on meter dial. Take several test readings.

12. RECORD on data sheet.

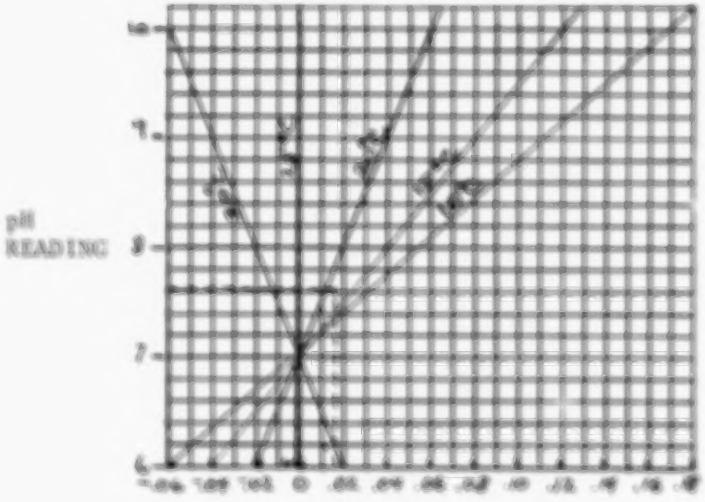
13. Rinse in water, dry with tissue, replace cap on electrode tip, slide sleeve back up over fill opening, and turn switch to off.

LABORATORY PROCEDURE:

A pH of a solution changes as temperatures change, and it may be necessary to make a correction to maintain accuracy. Interpolate the amount needed for your adjustment from the graph below.

Example: If your pH reading is 7.6 and the temperature is 18°, check the graph and you will note the adjustment figure to be approximately 0.015, or rounded to .02. Now add this to the 7.6 pH to obtain a pH reading of 7.62. RECORD on the data sheet.

pH CORRECTION GRAPH



ADJUSTMENT FIGURE

MER

pH Paper Method for Determining the pH of Water

Prepared by Bob Davis

MATERIALS TO BE TAKEN INTO THE FIELD:

1. Set of short range pH papers from pH values 6.0 - 9.5.
2. Water Sampler.

METHOD:

1. Obtain a sample of water and RECORD the depth.
2. Tear short (approximately 1") strips from the dispenser and dip into the sea water - remove quickly.
3. Compare the color to that on the color chart on the dispenser.
4. Repeat with each package until you are able to find a color which matches that on the dispenser.
5. RECORD this value as that of the pH of the water sample.

M E R

SALINITY

Prepared by Bob Davis

BACKGROUND

Sea water is a complex solution of dissolved minerals and organic materials. These chemicals are believed to have been added to the oceans over long periods of time as the rain waters flowing over the land leached dissolvable minerals and deposited them in the oceans. This buildup of minerals, commonly referred to as salts, is still occurring. Presently, the average salt concentration in a liter of water is 35 grams, or 35 parts per thousand (35 °/oo). Among the many minerals found in the ocean, the most common are:

Chloride $^-$	19.3
Sodium $^+$	10.7
Sulfate $^-$	2.7
Magnesium $^+$	1.3
Calcium $^+$	0.4
Potassium $^+$	0.4
Bicarbonate $^-$	0.1
Carbonate $^-$	0.007
Bromide $^-$	0.07

An important characteristic of all ocean waters is that the internal ratio of salts is constant throughout the world. This characteristic has been incorporated in many of the methods used to determine the salinity of water samples. By determining the concentration of one of the elements present, usually the chloride ion (Cl^-) by a simple titration procedure, one has only to multiply this value by a predetermined constant to arrive at the actual salinity. For example, if the water sample has 19 parts of chloride per 1000 parts of water, this will approximate a salinity of 35 °/oo.)

While salinity variation is small in the oceans, considerable fluctuation is common in estuarine situations. Often wedges of fresh water flow over the denser salt water, often extending into the sea. Ancient mariners often located larger rivers, such as the Amazon, by noting the low salinity of waters far out into the ocean.

Seasonal variations in fresh water flow results in great changes in local salinity and distribution of marine life forms. Many organisms which cannot tolerate either high or low salinities will die off when changes occur. Those forms,which through the evolutionary process have adapted to such changes, survive and become the dominant organisms in estuarine systems.

SALINITY: (Continued)

The San Francisco Bay - Delta - Estuary Complex is an example of a water system which typifies such a condition with varying salinity gradiants. As part of the MER MONITORING PROGRAM, careful measurements of the salinities at FIELD STATIONS will be made to learn more about the natural fluctuations of salinity. Correlation of the data obtained, with information concerning the life forms present, will shed light on the ecology of this complex.

Three major methods are generally employed for measuring salinities. The titration methods involve measurement of the chloride ion, and the calculation of salinity based on this value. Since salts conduct electricity, conductivity meters can be used. The greater the current, the "saltier" the water. Hydrometers can be used to determine the density of the water. Since salty waters contain more dissolved minerals, this technique may be useful, particularly where extreme accuracy is not required.

It is generally advisable to utilize several methods to check salinity in estuary waters because local runoff may result in waters in which the salt ratios are not as uniform as found in oceanic waters. The introduction of phosphates and nitrates into the waters from agricultural lands or industrial plants are common occurrences in this region. When readings from various methods do not agree, a frequent reason is that such "pollutants" are present.

As you monitor your assigned FIELD STATION, be careful so that your results do not suggest pollutants which may not exist. When you find repeated discrepancies between methods, you would begin to test for some of the more common minerals such as phosphates and nitrates which may be present.

MER

Determining the Chloride Ion Concentration and Salinity of Water - Mohr Method

Prepared by Bob Davis

MATERIALS

Reagents
Silver Nitrate Solution ($AgNO_3$)
Potassium Chromate Solution (K_2CrO_4) - 5%
Calcium Carbonate, powder ($CaCO_3$)
Burette, 25 or 50 ml
Erlenmeyer flask, 50 ml
2 Griffin Beakers, 50 ml
Water samples, top, 1 M and bottom
10 ml volumetric flask or pipette

METHOD:

1. Using a volumetric flask or pipette, remove 10 ml of water and place into the Erlenmeyer flask. *

2. Add a pinch (0.1g) of $CaCO_3$ using a clean spatula.

3. Fill a burette with silver nitrate solution to the zero mark. Slowly let the silver nitrate solution drain from the burette while *constantly* swirling the solution until a change in color is noted.

4. The end point is reached when the flaky precipitate which appears turns pinkish. This occurs at about the same time that the solution turns pinkish-orange. This can be a difficult end point to see, so titrate slowly at this point.

5. Calculate the volume of $AgNO_3$ used and RECORD it on the Data Sheet.

6. Repeat the above for each of the samples collected. It is standard practice to titrate at least two samples from *each* level.

7. If you are working in waters of low salinity, the volume of $AgNO_3$ used may be very small. If this is the case, increase the volume of the sample so that you will use a reasonable amount of $AgNO_3$. Why is this necessary?

8. Should you be working with waters which are acidic, it will be necessary to adjust the pH of the sample to neutral or slightly basic. Check with *Standard Methods* if this is the case.

METHOD (Continued)

Calculations

9. To calculate the chloride ion concentration in parts per thousand (°/₀₀) the following formula is used:

$$Cl^- \text{ in } °/_{00} = \frac{\text{Vol. of } AgNO_3 \text{ X Normality of } AgNO_3 \text{ X } 35.460}{\text{Sample Volume}}$$

Calculate the chloride concentration in ppm for each of the samples collected. The values obtained in the duplicate samples collected at the sample level should be very close on each trial. If you have difficulty in the beginning, run some extra samples and it will not take long to be able to obtain a series of results on the same sample which will be very similar.

RECORD the average results for the titrations of the samples obtained at each level.

10. Once the chloride ion concentration has been determined, the salinity can be calculated on the basis of Knudson's formula, as follows:

Salinity in °/₀₀ = 0.03 + 1.805 X Chlorinity

For the chlorinity values obtained from the samples at the three levels above, calculate the salinity and RECORD the values obtained on the Data Sheet.

*NOTE: Test pH of sample first. This titration works best if pH is in the 7-10 range. If your sample is not in this range, adjust the pH by adding Cl^- free 1N. H_2SO_4 or NaOH as necessary before proceeding.

M E R

Chloride Determination
Titametric Test (Delta)

INTRODUCTION:

The Delta Test involves using small quantities of sea water and reagents to determine the chloride concentration of sea water. In this method as with any method involving small amounts, one must be very careful with all measurements since small errors will become greatly magnified. Since this is a particularly important parameter, great care must be taken to insure that the data collected is accurate.

MATERIALS:

Water Samples: Surface, 1 meter and bottom
No. 8 Plastic Sample Tube
Filter
Filter paper
Reagent No. 3 (Phenolphthalein)
Reagent No. 5 (Standard Sulfuric Acid)
Reagent No. 1 (Chromate Indicator)
Reagent No. 2 (Standard Silver Nitrate)

METHOD:

1. If the sample is turbid due to the presence of sludge, rust, etc., it must be filtered prior to proceding with the test. If you are in doubt, run a test on two samples of water, one filtered and one not filtered. If your results differ, proceed with filtering all samples.

2. Rinse the No. 8 Plastic Sample Tube with the clear filtered sample and fill it to the mark.

3. Add 2 drops of Reagent No. 3 (Phenolphthalein). If the sample is red, add Reagent No. 5 (Standard Sulfuric Acid) drop by drop until the test sample is colorless. WARNING: Check with your instructor BEFORE adding the sulfuric acid. If the test sample is colorless on adding Phenolphthalein, do not add any acid.

4. Place 5 drops of Reagent No. 1 (Chromate Indicator) in the measured sample and add Reagent No. 2 (Standard Silver Nitrate) drop by drop with thorough mixing until a permanent reddish color results. Keep track of all drops added and always hold inverted dropping bottle in a vertical position. If held at

METHOD:

4. (Continued)
   another angle the size of the drops will vary. Each drop of Standard Silver Nitrate solution is equal to 10 parts per million of chloride.

5. RECORD the number of drops and calculate the salinity of the sample.

6. Repeat for each sample collected and RECORD.

M E R

La Motte Titration Method for Salinity

Prepared by Bob Davis

INTRODUCTION:

Use of the La Motte Method for Salinity Set as an alternative measurement of salinity (the investigator should read the general introduction to Salinity.)

MATERIALS:

La Motte Salinity Test Kit which includes:

Chloride Reagent 1
Chloride Titration Reagent 2
Chloride Titration Tube
Micro Burette
Pipette, uncalibrated
Distilled water

METHOD:

At the sampling station:

The sample will be collected at the time the water sampler is used in connection with the dissolved oxygen test.

LABORATORY TEST:

1. Place the hose from the water sampler into the bottom of an empty flask, and draw off approximately 10 ml.

2. Using the unmarked pipette, place three drops of the water to be tested in the Chloride Titration Tube. Carefully add distilled H_2O until the tube is filled to the 25 ppm line (6.0 ml capacity.) This is a one part to forty part (1:40) dilution.

3. Add three drops of the Chloride Reagent #1 to the sample in the Titration Tube. Insert the Titration Tube Cap and mix the contents. A yellow color will result.

4. The Micro Burette is filled with Chloride Titration Reagent #2 in the manner described below. Insert the Micro Burette in the center hole of the cap.

   TO USE MICRO BURETTE: Fit Micro Burette into cap of Chloride Titration Reagent #2. Invert and fill the Micro Burette by withdrawing the plunger. Fill until the burette reads zero. Turn the bottle right side up and remove the burette.

LABORATORY TEST: (Cont.) Place the burette in the hole of the Titration Tube Cap. Depress the plunger to expel the Chloride Titration Reagent #2. A slight rotating or twisting motion permits the plunger to move smoothly. Agitate the Titration Tube so that each addition of the Titration Reagent is thoroughly mixed with the sample.

5. Continue to titrate until the color of the mixture changes from yellow to pinkish-brown.

6. The results of the test are read from the scale on the barrel of the Micro Burette. Each major division on the scale is equal to 1.0 ppt. (parts per thousand) and each minor division is equal to 0.2 ppt.

7. RECORD your results on the data sheet.

M E R

Measuring Turbidity

Prepared by Bob Davis

BACKGROUND:

The production of the phytoplankton, the "producers" of the ocean is limited by light penetration. Here in San Francisco Bay and the entire estuary, light penetration generally is quite low. This condition results from two main causes. First, there is often a large amount of silt in the water which prevents light penetration and second, in areas rich in nutrients, high growth of plankton at the surface reduces light penetration to lower depths. The "clarity" of the water is referred to as turbidity.

There are several methods for measuring turbidity. Three methods which we will employ are the: 1) Secchi Disk, 2) a direct reading photometer, and 3) a Millipore technique. Each method has its advantages and limitations.

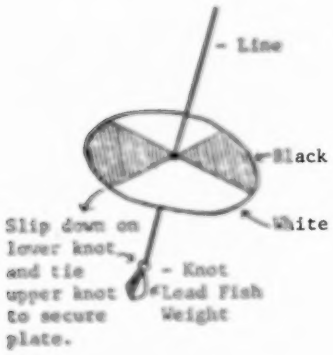
MER

Secchi Disk Technique for Measuring Turbidity

Prepared by Bob Davis

MATERIALS:

Secchi Disk



METHOD:

Field Procedure

1. Lower the Secchi Disk into the water and RECORD the distance from the surface of the water to the disk at the point at which the disk just disappears from view. Raise the disk and RECORD the distance from the surface of the water to the disk in meters.

2. Determine the average distance between these two values. RECORD

3. Repeat the procedure two more times as above. If the averages for the three trials are different, average the results of the three trials.

Laboratory Activity

The value you have determined in the field represents the lowest level of visible light returning back through the water. In reality, light penetration is deeper.

To compare measurements obtained throughout the world, oceanographers convert the value (average length) into what is called the Turbidity Factor. This value is the relationship between the distance the light traveled through the waters at a given station to the distance it would have traveled through distilled water under similar light intensity.

To calculate the Turbidity Factor, use the following formula:

$$\text{Turbidity Factor} = \frac{1.7}{\text{Average Distance}}$$

M E R

Photometric Technique For Measuring Turbidity (Delta)

INTRODUCTION:

The use of a photometer for measuring turbidity provides a simple method particularly adapted for turbid waters. Since the waters of the Bay Complex are frequently turbid due to the large amounts of fine silt being carried, this technique is an important one to include when monitoring for this parameter. The final readings will be expressed as ppm silicia, a commonly used standard.

EQUIPMENT:

Delta Photometer
Filter holder with blue dot
2 - No. 50-T Test Cells
Distilled or deionized water
Turbidity Graph for the meter
Water samples from the surface, 1 M depth and bottom.

METHOD:

1. Insert the filter holder all the way into the slot so that the *blue dot* faces upward.

2. Fill a clean, dry No. 50-T Test Cell to the mark with sample to be tested, and cap the cell. The No. 50-T Test Cells must be completely clean and free from film, lint, fingerprints, etc. Do *not* use soap when washing the cells.

3. Fill a second clean, dry test cell to the mark with clear, turbidity-free distilled or deionized water, for use as a blank. Cap the cell.

4. Insert the cell with the clear water into the photometer. Press the button switch, and turn the knob to set meter to 0. Then promptly release button switch.

5. Remove the cell and replace it with the cell containing the turbid sample. Press the button, and when the meter comes to rest, read meter value. RECORD this value on the Data Sheet. Do not touch knob adjustment in this step. To extend the life of the battery, release the push button promptly, as soon as needle comes to rest and reading has been taken.

PHOTOMETRIC TECHNIQUE FOR MEASURING TURBIDITY (Continued)

METHOD (Continued)

7. Clean the cells and caps thoroughly after use, and allow to dry. Do not use soap.

8. Repeat the procedure for the remaining samples and RECORD the results on your Data Sheet.

MER

Millipore Technique for Measuring Turbidity

Prepared by Bob Davis

METHOD:

Materials to take to the sampling station:

One 250 cc bottle for each of the depths to be sampled.

At the sampling station:

A sample of the water should be taken at each of the following depths: surface, 12" and 24". Simply uncap the jar when you are holding it below the surface at each of these depths.

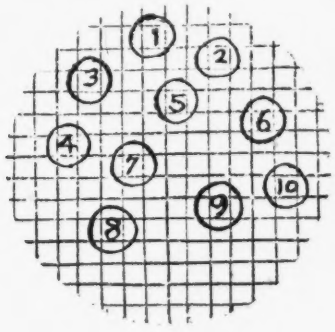
Laboratory tests:

In this test a Millipore filter of 0.45 microns will be used, thus allowing any particle larger than 0.45 microns to be trapped on the surface of the filter. A random sampling of these particles will be made to obtain a measure of the total suspended particles in our sample.

1. To the receiver flask attach the filter base and on top of this place the Millipore filter. Attach the 250 ml funnel.
2. Fill the funnel to 250 ml and put on the cover. (Make sure that you shake the sample in the collecting bottle well before pouring out the water.)
3. Attach the vacuum system which consists of a plastic syringe, a length of rubber tubing, and a two-way valve. One end of the tubing carries a small nylon adapter. Slip the other end tightly over the side vent of the valve, and attach the valve to the syringe. Insert the nylon adapter into one side arm of the receiver flask and close the other side arm with a gum rubber cap. When the syringe plunger is now worked, air will be drawn out of the receiver flask and vented through the valve outlet. This will fill your receiver flask with filtered liquid.
4. When all liquid has filtered through into the receiver flask, remove the filter from the container by use of forceps.
5. Place on blotting paper, face up and allow to dry *completely*. If you procede to step 7 and add oil to the wet filter, it will not become translucent.

METHOD: (Millipore Technique - continued)

6. When dry, take a ballpoint pen and circle the samples as shown below. Be careful to make your circles large enough so they do not come near any line on the square you will count.



7. Float your filter on a thin layer of immersion oil in a clean petri dish. This will render it transparent for microscope use.
8. Transfer the filter to a glass slide for counting the particles in each sample square.
9. Use low power of your microscope. If a part of a particle touches the top or left hand line of the square, count it; if it touches the bottom or right hand line of the square, do NOT count it. Why is this necessary? Tabulate the number of particles in each square. RECORD your results on your data sheet.
10. This count is then multiplied by a factor to derive the count which would have been found had the entire surface of the filter been scanned.

    The formula is:

    $$\frac{145}{\text{10 grid squares counted}} \times \text{number of particles} =$$

    the estimated number of particles on the filter.

11. RECORD this figure as the total suspended particles/250 ml of water.
12. Repeat for each level.
13. RECORD your results.
14. To express your results in terms of particles per liter, multiply the values obtained by 4.
15. Occasionally, you will find it difficult to count particles if the concentration is too high. Should this occur, use a smaller water sample, i.e. 25 ml. When you complete step 11, your count would be the number of particles/25 ml. To express this in particles /l, multiply the answer by 40, the dilution factor.

M E R

TEMPERATURE DETERMINATION

Prepared by Bob Davis

INTRODUCTION:

Special devices,including reversing thermometers, electric resistance thermometers and bathythermographs, are used in determining temperatures at any depth of water. However, for our purposes, an ordinary mercury thermometer serves well. Temperatures of water are to be taken at each depth a water sample is taken as well as temperature readings of the air taken at the waters' surface and in a standing position.

MEASUREMENT:

In order to be as accurate as possible, all thermometers should be corrected to agree with the readings of the "standard" maintained in the laboratory.

MERCURY THERMOMETER TEMPERATURE READINGS

METHOD:

Materials to be taken to sampling station:

Mercury thermometer 10-80°C adjusted to agree with standard in the laboratory.

At the sampling station:

1. Take temperature reading by holding thermometer at breast height. Hold by the tip in this position for two minutes or until the mercury column is stabilized. RECORD on data sheet.
2. Hold thermometer about an inch above the water surface for two minutes or until the mercury column is stabilized. RECORD on data sheet.
3. RECORD the temperature reading each time the water sampler is recovered and the depth at which the sample was taken. As soon as the sampler is brought to the surface, remove the rubber stopper in the top cap, and insert the thermometer with the corresponding rubber stopper. Allow to stand for two minutes before reading. When removing to read, make sure the mercury tip remains in the container while reading.

NOTE: BE SURE TO READ TEMPERATURE TO THE NEAREST .1 DEGREES CENTIGRADE.

Example: 22.3 degrees centigrade

MER

Nitrate and Nitrite Nitrogen Determination
Colorimetric Method (Delta)

INTRODUCTION:

In aquatic ecosystems nitrates play a major role in determining the productivity of the waters as they are necessary raw materials for the growth of plants. Without the necessary minimum amounts of nitrates and nitrites, plant growth would not be possible and the producers, green plants, would not develop thereby supplying the necessary food and oxygen to support the animal forms.

A major problem in maintaining water quality in regions such as ours where human population density is high around the Bay and much agriculture occurs in the delta region is that excess nitrogen compounds are released into the waters. Agricultural runoff from fields receiving fertilizers and treated sewage waters frequently contain high concentrations of dissolved nitrates. When this occurs and similarly high concentrations of phosphates are present, abnormally high algal growth results. Unfortunately, water can only dissolve a limited amount of the oxygen produced by photosynthesis. During the evenings, the plants and animals quickly consume the oxygen in the waters and death occurs from oxygen depletion. The decomposition of the dead organisms with the production of poisonous gasses further reduces the variety of life present in the waters and renders it of poor quality. This phenomena of oxygen depletion as the result of forced algal growth is called eutrophism.

The procedure being followed involves three steps. First, the total nitrate and nitrite concentrations are determined in Part I. Then, in Part II, the nitrite $(NO_2)^-$ concentration is determined. The nitrate $(NO_3)^-$ concentration, Part III, is simply calculated by subtracting the results in Part II from the value determined in Part I.

As part of the MER Monitoring Program, cyclic variations in the concentrations of nitrogen compounds in local waters will be determined.

NITRATE AND NITRITE NITROGEN DETERMINATION (Continued)

MATERIALS:

Water sample
Delta Photometer
4 Test Cells No. 50-T
No. 35 Scoop (0.5g)
Reagent No. 46 (Nitrate-Cadmium Reagent)
No. 33 Scoop (0.2g)
Reagent No. 47 (Nitrite Reagent)
Distilled water

PART I

TOTAL NITRATE - NITRITE NITROGEN DETERMINATION

METHOD:

1. Insert the filter holder all the way into the slot with the single green dot facing upward.

2. Fill the No. 50-T Test Cell to the mark (10 ml) with the sample to be tested. MAKE CERTAIN THAT THE TEST CELL HAS BEEN SCRUPULOUSLY CLEANED AND DRIED WITHOUT USING SOAP. If your sample is colored or turbid, see the instructions at the end of Part I. (Note A)

3. Prepare a reagent blank by filling a second clean No. 50-T Test Cell with distilled or deionized water to the 10 ml line.

4. Using the No. 35 Scoop (0.5g) add 1 level scoopful of Reagent No. 46 (Nitrate-Cadmium Reagent) to <u>each</u> cell. Cap the cells securely, note the time, and shake both cells vigorously <u>for 1 full minute</u>.

   IMPORTANT: Always replace the cap promptly and tightly on the bottle containing Reagent No. 46 to prevent the powder from picking up moisture from the air. The bottle contains a capsule of dessicant to absorb any excess moisture. <u>Keep the scoop clean and dry</u>.

5. Allow the cells to stand 5 minutes after shaking for color development. DO NOT DELAY LONGER THAN 12 MINUTES AFTER STEP 4.

6. Insert the cell containing the "blank" into the photometer. Press the button switch and turn the knob to set the meter to 0. Then, promptly release the switch.

NITRATE AND NITRITE NITROGEN DETERMINATION (Continued)

7. Immediately remove the cell blank and replace it with the cell containing the treated sample. Press the button and when the meter needle comes to rest, read and RECORD the meter value. Do not touch the knob adjustments at this point. To protect and extend the life of the meter, do not hold the button any longer than is necessary to obtain a reading.

8. Immediately remove the cell and clean the sample and blank cells and caps thoroughly, rinse with distilled water and dry. Do not use soap.

9. To determine the concentration of nitrates, use the Nitrate/Nitrite Graph prepared specifically for the meter you are using. RECORD this value on the Data Sheet as Combined Nitrate/Nitrite Nitrogen.

10. The range of the test is from 0 - 2 ppm nitrogen (N). If your sample should contain nitrates in abnormally high concentration, a simple modification is possible. For example, you may take 10 ml of the original sample and dilute it with 10 ml of distilled water. The result will be a solution containing 1/2 the original nitrogen. After you have performed the above procedure on the diluted sample, multiply the results by the *Dilution Factor* and RECORD. In the example above, the *Dilution Factor* would be 2.

ppm = parts per million

NOTE A: If the sample is colored or turbid, fill one No. 50-T Cell with a sample and another No. 50-T Cell with clear water. Set the meter to 0 with the clear water, replace the cell containing clear water with the cell containing the turbid or colored water and RECORD the meter reading. Convert this reading to equivalent ppm N from the graph and subtract this value from the results obtained in the procedure (step 9 to 10.)

PART II

NITRATE DETERMINATION

METHOD:

1. Filter holder should be inserted all the way into slot, so that 1 green dot faces upward.

2. Fill a clean No. 50-T Test Cell to the mark (10 ml) with the sample to be tested.

NITRITE DETERMINATION (Continued)

3. Simultaneously prepare a regent blank by adding 10 ml of nitrite-free or distilled water to a second No. 50-T Test Cell.

4. Using the No. 33 Scoop (0.2g) add 2 level scoopfuls of Reagent No. 47 (Nitrite Reagent) to each cell. Cap the cells securely and shake both cells to dissolve the reagent. Note the time and let stand for 6 minutes for color development. DO NOT DELAY TESTING LONGER THAN 12 MINUTES AFTER THIS STEP.

   IMPORTANT: Always replace the cap promptly and tightly on the bottle containing Reagent No. 47 (Nitrite Reagent) to prevent the powder from picking up moisture from the air. Bottle contains capsule of dessicant to absorb moisture. Keep measuring scoop clean and dry.

5. Insert the cell containing the "blank" into the photometer. Press the button switch and turn the knob to set the meter to 0. Then promptly release the switch.

6. Immediately remove the cell, and replace it with the cell containing the treated sample. Press the button and when the meter needle comes to rest, read meter value. Obtain test results in ppm Nitrogen directly from the Nitrate/Nitrite graph. RECORD the value on the Data Sheet. Do not touch the knob adjustment in this step. To extend the life of the battery, release the push button promptly, as soon as needle comes to rest and reading has been taken. Clean the cells and caps thoroughly after use, rinse with distilled water. Do not use soap.

7. The range of the test is 0-2 ppm Nitrogen (N). To measure higher values, dilute the test sample with distilled or deionized water, and multiply the result by the Dilution Factor. For example, if 10 ml is diluted to 20 ml and 10 ml of diluted sample is used in the test, multiply result by 20/10 or 2. The range of test is now 0-4 ppm N.

PHOSPHATE DETERMINATION
COLORIMETRIC METHOD (DELTA)

INTRODUCTION:

The role of phosphates $(PO_4)^{=}$ in aquatic ecosystems is of great importance, serving as a necessary raw material for plant (algae) growth. While traces of phosphates are necessary, excess phosphates cause excessive plant growth which can lead to problems of eutrophism (see introduction to Nitrate and Nitrite Nitrogen Determination.)

The following method is designed to determine the amount of inorganic phosphate present in a water sample. In the Ortho-Phosphate test, you will determine the amount of inorganic phosphate in "ortho" form, that is, where a single phosphate group $(PO_4)^{=}$ is attached to an element. In Part II, the total available phosphates will be measured. Polyphosphate determination, the condition where more than one $(PO_4)^{=}$ group is associated with an element, is determined by simply subtracting the concentration of Ortho-Phosphate from the Total Phosphate concentration which will be determined.

Monitoring for phosphates at the FIELD STATIONS is important in determining the normal phosphate fluctuations occurring in the water complex.

MATERIALS:

Water samples from surface, 1 meter depth, and bottom
Delta Photometer
Funnel
Filter Paper
50 ml Griffin Beaker
4 - No. 50-T Test Cells
Reagent No. 41 (Sulfuric Acid 15%)
Reagent No. 39 (Ammonium Molybdate, 2.5%)
Reagent No. 40 (Stannous Chloride)
Erlenmeyer Flask, 50 ml (No. 42)
Hot Plate

PART I

ORTHO-PHOSPHATE DETERMINATION

METHOD:

1. Filter about 25ml of the water sample collected the Griffin beaker.

2. Fill a No. 50-T Test Cell to the mark (10 ml) with a filtered sample.

3. Simultaneously prepare a reagent blank by adding 10 ml of phosphate-free or distilled water to a second No. 50-T Test Cell.

4. Add 10 drops of Reagent No. 41 (Sulfuric Acid 15%) to each and mix. Hold bottle vertically, with tip facing down, when counting drops. If this is not done carefully, drop sizes will vary.

5. Add 6 drops of Reagent No. 39 (Ammonium Molybdate, 2.5%) to each and mix.

6. Add 1 drop of Reagent No. 40 (Stannous Chloride) to each and mix.

7. Allow to stand 15 minutes for full color development.

8. The filter holder should be inserted all the way into slot of the photometer so that the yellow dot faces upward.

9. Insert the cell containing the "blank" into the photometer. Press the button switch and turn the knob to set meter to 0. Then promptly release switch.

10. Remove the cell and replace it with the cell containing the treated sample. Press the button and when the meter needle comes to rest, read meter value. Obtain the test results in ppm phosphate. RECORD the value on the Data Sheet. Do not touch knob adjustment in this step. To extend the life of the battery, release push button promptly, as soon as the needle comes to rest and the reading has been taken. Clean cells and caps thoroughly after use, and allow to dry.

ORTHO-PHOSPHATE (Continued)

11. The range of test is 0-5 ppm $(PO_4)^{=}$. To measure higher values, dilute the test sample with distilled or deionized water, and multiply result by Dilution Factor For example, if 10 ml is diluted to 20 ml, multiply result by 20/10, or 2. However, do not forget to use only 10 ml in the test.

PART II

TOTAL PHOSPHATE DETERMINATION

METHOD:

1. Measure 10 ml of filtered sample, using a No. 50-T Test Cell, into the No. 42, 50 ml Erlenmeyer Flask.

2. Add 10 drops of Reagent No. 41 (Sulfuric Acid, 15%) and mix.

3. Allow acidified sample to boil gently for 90 minutes adding distilled water to keep the volume between 7 and 10 ml. In the field, an inexpensive "Sterno-Type" Stove can be used. See Note A.

4. Cool the sample and transfer it to a No. 50-T Test Cell. Dilute with distilled water to the 10 ml mark.

5. Simultaneously prepare a reagent blank by adding 10 ml of phosphate-free or distilled water to the mark of a No. 50-T Test Cell and add 10 drops of Reagent No. 41 (Sulfuric Acid).

6. Add to each, while mixing, 6 drops of Reagent No. 39 (Ammonium Molybdate) and 1 drop of Reagent No. 40 (Stannous Chloride Reagent). Let stand for 15 minutes for full color development.

7. Continue as directed in procedure for Ortho-phospate, beginning with Step 6.

8. RECORD the total phosphate value on the Data Sheet.

PART III

POLYPHOSPHATE DETERMINATION

METHOD:

To determine Polyphosphate, subtract the result obtained in Procedure A (Ortho-phosphate), from the result obtained in Procedure B (Total Phosphate). The difference represents Polyphosphate, expressed in terms of Phosphate $(PO_4)^{=}$.

	Total Phosphate	_____ ppm
—	Ortho-Phosphate	_____ ppm
	Polyphosphate	_____ ppm

RECORD this value on the Data Sheet.

NOTE A -- Some samples may require less boiling time. This can easily be determined by running duplicate samples, using a 90 minute and a 30 minute boiling cycle and comparing the results on the colorimeter. If readings are the same, then the 30 minute boiling time may be employed for samples of similar composition.

NOTE B -- The Stannous Chloride method has been adopted because of its sensitivity in the lower phosphate ranges. The minimum detectable concentration is approximately 0.02 mg/L of $PO_4^{=}$. To avoid contamination from interfering substances, it is recommended that all glassware used in this test be thoroughly cleaned with No. R-95 Acid Dichromatic Solution and rinsed several times with demineralized distilled water. Do not use soap, detergents or glassware cleaners, as they may leave a film of phosphate on the glass, which would cause errors.

M E R
MONITORING PROGRAM

CHAPTER II

TECHNIQUES FOR STUDYING POPULATIONS

in the

SAN FRANCISCO BAY - DELTA - ESTUARY COMPLEX

M E R

Population Studies

BACKGROUND:

The study of populations in their natural habitats usually requires a bit of ingenuity on the part of the investigator. *The objective of the portion of MER, simply stated, is to measure the numbers of an organism in a given area--a field station--at a specific time.* The techniques employed to accomplish this objective will vary depending on the substrate at the station, the kinds of organisms being inventoried, and the accessability of the area being studied. If one were attempting to study a fish population, a technique employing a seine net or boat trawl might be employed. However, if one were studying worm or clam populations, collection techniques which could be used may include special dredges or bottom grabs in deep waters whereas core samplers or shovels may be used on sandy or muddy beaches.

The following techniques are commonly employed by those attempting to study populations. Depending upon the nature of the station being monitored as part of the general monitoring program, one or more methods will be selected for use at a given station.

When your first monitoring is completed, you will be able to calculate the number of organisms of a specific kind at the station on a specific day and time. Further samplings should indicate that populations are not constant during the year. Some resident species may totally disappear for periods and suddenly return later; others may remain fairly constant during the year. Variations in the numbers of a given species are natural phenomena frequently related to seasonal changes in the properties of the water, i.e., temperature and salinity. In many cases the organisms you will encounter have not been carefully studied and little is known about their life cycles, their natural seasonal population fluctuations, etc. Data which you collect will be correlated with many variables in an attempt to learn more about the organisms which are found in this bay-delta-estuary complex. In addition, long term changes in "water quality" which appear inevitable, should result in the appearance or disappearance of organisms during the years to come. *Careful records of conditions today and the populations present can serve as a base line against which these anticipated changes can be compared.*

MER

Studying a Plankton Population

BACKGROUND:

Plankton is a term used to describe the smaller organisms which are found in water and are trapped in fine mesh nets which are pulled through the water. Within a sample will be found organisms representing members of both the plant and animal kingdoms. The plant portion is generally referred to as phytoplankton and the animal portion as the zooplankton. The phytoplankton are the fundamental producers, the organisms which are responsible for the production of oxygen and the conversion of minerals into organic material - food, upon which all life depends. The zooplankters are consumers which prey on the phytoplankters and upon each other. This group includes such diverse forms as protozoa and the numerable larval stages of invertebrates and even some fish. All these plankters are preyed upon by filter feeders such as barnacles, clams and certain fish. The plankters form a most important part of the food-web of a water system supplying food and oxygen for the larger life forms. Their populations vary considerably during the year. As the waters warm, many free swimming larval forms such as the algae, the larvae of various invertebrates and smaller fish may become dominant organisms. To the aquatic biologist, an analysis of not only the density of plankters, but also knowledge of the fluctuations of the various kinds of plankters offers insight into biological activity in the waters.

Several activities are included which will shed information on our water complex if they are systematically done over a period of time. As part of Project MER's Monitoring Program, plankton studies are included. The data from these studies will help us to better understand our water problems.

M E R

Measuring the Density of Plankton

INTRODUCTION:

The measuring of plankton density, the number (or weight) of organisms in a unit volume of water, i.e., number per cubic meter, is a realatively easy technique and one which can provide the biologist with important information concerning the productivity of waters. Two methods are outlined below and it is important that both methods be carried on simultaniously. The first method involves determining the density in weight g $/M^3$; the second method involves determining the density in numbers of organisms $/M^3$. Why do you think that two methods should be employed?

METHOD I

THE DETERMINATION OF THE DENSITY OF PLANKTON IN WEIGHT g $/M^3$

MATERIALS:

Plankton net with size 10 mesh
Towline
Balance accurate to 0.01 g
Plankton collection bottles with caps (6)
Measured length of rope, 5 meters
Millipore Filtration Apparatus
H. A. Grid Filters with a 0.45μ pore size
Formaldehyde in dropper bottle
Net modifications illustrated in Fig. 1 and 2
Meter stick or rope knotted at 1/2 M intervals, 3 M long

FIELD PROCEDURE:

1. Mark off a known distance parallel to the shore which you are sampling.

2. Lower the net into the water just under the surface and pull the net at a uniform rate with sufficient speed into the flow of the tide. Make certain that you are moving faster than the tide and the net opening is perpendicular to the surface of the water.

3. At the end of the measured distance, reverse your direction and walk back to where you started. Try to keep the net at the same depth and moving at the same rate. (This sounds easy but will require some practice.) If you should stir up the mud, you may have to move a short distance away to obtain a sample

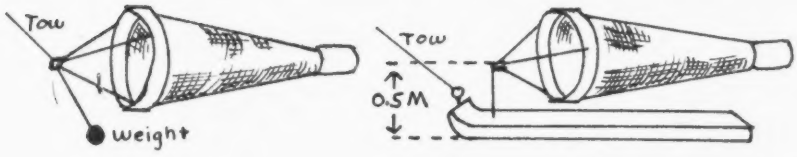
FIELD PROCEDURE:

3. (Continued) of clear water. You will have problems if the net contains mud or sand since you will find it difficult to separate these particles from the plankton.

4. Remove the bottle from the net, cap it, and RECORD exactly where the sample was taken, the depth, and time in a field notebook. Be sure to identify the sample bottle with a number for reference later.

5. Repeat the same procedure and obtain 2 samples at each depth. You will need two surface samples, 2 at a depth of 1/2 meter and 2 at a recorded depth just above the bottom. Use a measuring device for accuracy.

   NOTE: In swift moving waters you may have difficulty maintaining your net at a uniform depth and have to develop your own solutions in overcoming this obstacle. As a suggestion, you may place a weight at the apex of the cone formed where the net is attached to the towline. If you do this, be careful the opening of the net is kept perpendicular to the surface of the water. If the water is deep, it will be necessary to mount the net on a sledge and pull the sledge along the bottom.



6. Determining the Volume of Water Sampled, measure the opening of the net in meters. Using the following formula, determine the total volume of water which passed through the net.

   (πr^2) (total distance traveled) = volume of water sampled in M^3

RECORD this value and your calculations on your data sheet.

EXAMPLE:

Total Distance of Net Tow	*10 M M*
Diameter of the Net in Meters	*0.24 M*
Volume of Water Sampled in M^3 =	
$(\pi\ r^2)$ (Tow Distance) =	
$\left(\frac{22}{7} \cdot 0.12^2\right) \times (10\ M)$ =	
$\left(\frac{22}{7} \cdot 0.0144\right) \times (10\ M)$ =	$0.4526\ M^3$

LABORATORY PROCEDURE:

7. Assemble a Millipore Filtration Apparatus. Pour some water through the filter to wet it. Pump it to evacuate the water from the top chamber.

8. Remove the filter carefully with forceps and weigh it carefully on the balance. RECORD this weight in Grams on your data sheet. Reassemble the Millipore Filtration Apparatus using the same filter.

9. Pour the sample into the Millipore Filtration Apparatus. Rinse out the sample bottle and add this water to the sample in the filtration apparatus.

10. Using the plunger, draw the sample into the bottom 1/2 of the filtration apparatus. You should wash down the sides of the upper container as necessary to force the plankton onto the filter.

11. Remove the filter and weight it. RECORD in Grams on your data sheet.

12. Calculate the weight of the plankton sample as follows:

Weight of the wet filter + Plankton = _____ g
(-) Weight of the wet filter = _____ g

Weight of the Plankton in the sample= _____ g

EXAMPLE:

Weight of the wet filter + Plankton	= *6.06 g*
- Weight of wet filter	= *1.01 g*
Weight of the Plankton in the Sample	= *5.05 g*

13. The final calculation involves equating the weight of the sample obtained in each tow to the weight which would have been collected had one sampled exactly one M^3 of water.

$$\frac{\text{Weight of Sample(g)}}{\text{Volume of Sample } (M^3)} = \frac{\text{Density in Weight of Organisms(g)}}{M^3 \text{ of Water}}$$

EXAMPLE:

$$\frac{5.05\ g \text{ (Wt. of Sample}}{0.4526\ M^3 \text{ (Vol. of Sample)}} = 11.16$$

Density of Plankton in Grams/M^3

14. Calculate the density of one sample taken at each depth and RECORD on your data sheet.

MER

METHOD II

DETERMINING THE DENSITY OF PLANKTON IN NUMBERS/M^3

MATERIALS:

Previously collected plankton samples
Millipore Filtration Apparatus
H. A. Grid Filters with a 0.454 pore size
300 ml of isotonic saline solution
Forceps
Immersion oil
Petri dish
1 - 10 ml, 1 - 100 ml graduated cylinders
Monocular microscope

FIELD PROCEDURE:

1. The second samples collected previously (see METHOD I) is reserved for this procedure.

LABORATORY PROCEDURE:

2. Pour the plankton sample into a 100 ml graduated cylinder.

3. Rinse the bottle several times with an isotonic solution to insure that all plankters which might adhere to the side of the container are transferred into the graduated cylinder.

4. Fill the graduated cylinder to the 100.0 ml mark.

5. Shake the cylinder well to insure that the plankton are uniformly distributed and quickly pour out 1.01 ml into a 10 ml graduated cylinder. This 1.0 sample should contain 1/100 the number of plankton which you collected in the tow.

6. Prepare a Millipore Filtration Apparatus using a Type H. A. Grid filter with a 0.454μ pore size.

7. Add approximately 100 ml of isotonic salt to the top portion of the filtration apparatus.

8. Pour the 1.0 ml sample into the filtration apparatus. Rinse the 10.0 ml graduated cylinder with isotonic salt solution to make certain that all the plankton are transferred into the apparatus.

9. Swirl the salt solution and sample carefully to insure uniform mixing.

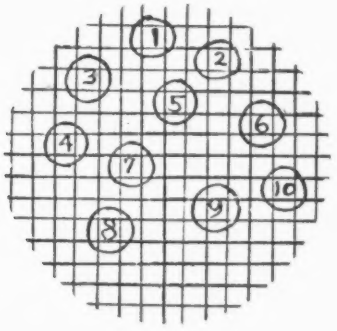
10. Using the syringe, force the water into the lower part of the filtration apparatus.

11. Rinse down the sides of the upper half of the filtration apparatus with approximately 50 ml of salt solution to insure that no plankton adhere to the sides.

12. Again using the plunger, force the solution through the filter leaving the plankton evenly distributed on the filter surface.

13. Open the filtration apparatus and carefully remove the filter using the forceps. Place the filter on a towel or blotting paper, plankton side up, to dry. This should take approximately 5 minutes.

14. When dry, take a ballpoint pen and circle the samples as shown below. Be careful to make your circles large enough so they do not come near any line on the square you will count.



15. Float your filter on a thin layer of immersion oil in a clean petri dish. This will render it transparent for microscope use.

16. Transfer the filter to a glass slide for counting the particles in each sample square.

17. Use low power of your microscope. If a part of an organism touches the top or lefthand line of the square, count it; if it touches the bottom or righthand line of the square, do NOT count it. Why is this necessary? Tabulate the number of plankton in each square. RECORD your results on your data sheet.

NOTE: At this point, you may observe that the plankton are so densely packed that you cannot obtain an accurate count. If this is the case, it will be necessary to go back to step 4. Decide how much greater a dilution will be necessary to provide you with a sufficiently diluted sample. If you wish to dilute by 10% more, take a well mixed 10 ml portion of the previously diluted plankton sample you prepared and place it in another 100 ml graduated cylinder -- add saline to the 100 ml mark. The *Dilution Factor* you will now have to use for further calculations will be 1,000.

If you find that there are too few organisms on the filter, go back to step 5 and proceed. Instead of taking a 1.0 ml sample from the 100 ml graduated cylinder take the necessary amount which will give you a reasonable number of organisms to count, i.e., if you wished to increase the number 10 fold, take 10 ml from the 100 ml graduated cylinder and add this to the filtration apparatus. If 10 ml were taken, the *Dilution Factor* which you will later use will be 10.

18. The number of plankton counted are then multiplied by a number to calculate the count which would have been found had the entire surface of the filter been scanned.

This formula is:

$$\frac{145}{\text{10 grid squares counted}} \text{ X Number of Plankton} =$$

the number plankton on the filter

19. RECORD this figure as the total number of plankton on the filter

EXAMPLE:

$\frac{145}{10}$ X 22 = 319 Plankton on Filter

20. Knowing the number of plankton on the filter, it is easy to estimate the number in the original sample by multiplying the number on the filter by the *Dilution Factor*.

Number on the filter X Dilution Factor = Number of Plankton in the sample

EXAMPLE:

319 X *100* = *31,900* Plankton in Sample

21. Assuming that you took two identical samples of plankton in the field, you will have the calculations (METHOD I, step 6) of the volume of water sampled. Unless you sampled a different volume, you can use this figure to determine the Density of Organisms in Numbers/M^3. If not, you will have to make separate calculations.

22. To calculate the Number of Plankton/M^3, simply divide the number of plankton in the sample by the volume of water sampled originally.

Formula:

$$\frac{\text{Number of Organisms in Sample}}{\text{Volume of Sample in } M^3} = \frac{\text{Number of Organism}}{M^3}$$

EXAMPLE:

$$\frac{31,900}{0.4526\ M^3} = 70,481 \text{ Plankton/}M^3$$

23. Calculate the Density of Plankton in each of the three samples at different depths as indicated and RECORD on your data sheets.

M E R

Surveying Mud Flat or Sandy Beach Population

INTRODUCTION

The following steps are normally followed when determining populations.

1. Identification of specific areas at the sampling station(s) to be studied. (Field)
2. Collection of uniform samples. (Field)
3. Separation and identification of organisms. (Laboratory)
4. Tabulation of numbers of each species. (Laboratory)
5. Determination of species biomass where appropriate. (Laboratory)
6. Calculation of populations in numbers/unit area. (Laboratory)

MATERIALS:

1. Compass
2. 3' carpenter's level
3. Light weight rope, 50'
4. Transit or sighting apparatus
5. Bio-Core Sampler
6. Thermometer, (Shielded type preferred)
7. Containers to carry sample cores
8. Finger bowls and dishes
9. Dissecting needles, probes and droppers
10. Dissecting microscope

METHOD:

1.0 Identification of the specific areas at the sampling station. The identification of the specific area to be sampled must be done with great care for two reasons. First, anyone going to the station a second time must be able to find it to enable continuous monitoring. Second, if the data collected is to have value, it is essential that all distances are carefully calculated.

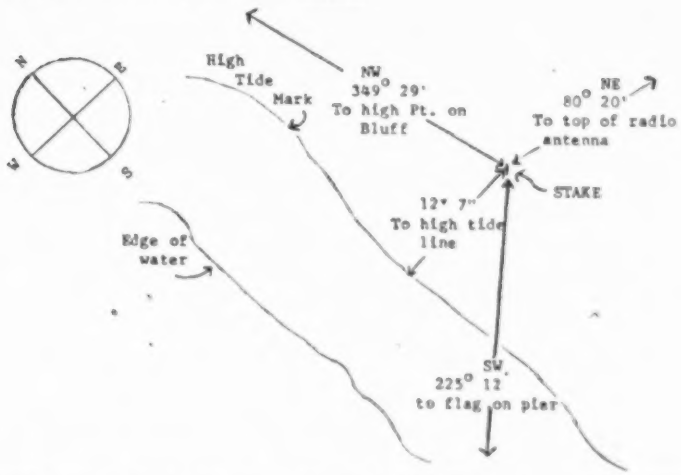
1.1 Locate a tree or permanent object near the water which will serve as a "bench mark." If such an object is not present, a stake will have to be used. When a stake is used, you must make certain that anyone can relocate it if someone should remove it or future monitoring at the

METHOD: (Population Studies)

1.1 (Continued) station will not be possible. DRAW an accurate diagram of the area for future reference. See Figure 1.

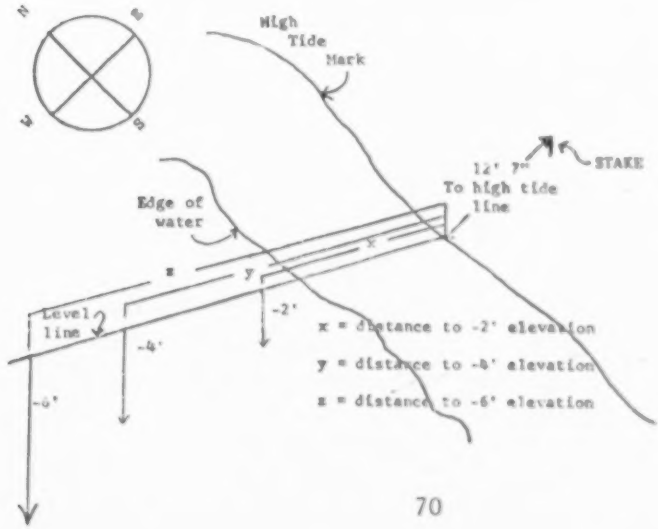
1.2 Attach a rope to the "bench mark" and run a line to the high water line. This high water mark can be easily located by examining the beach for the line of debris which is left at the high water point. If rocks are nearby, discoloration usually indicates this elevation. This point will serve as the "high water" collection area. Measure the distance from the bench mark to this point. RECORD. See Figure 1.

Figure 1. Locating a Stake EXAMPLE



1.3 Continue the line out toward the water. The next area will be located at that point where the elevation has dropped 2 feet. To determine the drop accurately, use a carpenter's level. RECORD the distance to this point from the bench mark to facilitate future sampling. See Figure 2.

Figure 2. Locating the Sampling Areas at the Station



METHOD: (Population Studies, Continued)

1.4 Repeat the above step and determine the distance to the point where the water elevation has dropped 4 feet. If possible, repeat to the 6 foot drop. (Note: Since tidal fluctuations vary within the bay-delta-estuary complex, it might not be possible to establish as many points for sampling as one goes "upstream." If the tidal fluctuations are small, sample at intervals of one foot drops in elevation. As a rule-of-thumb, you should be able to establish at least three specific locations at a station from high to low water. See Figure 2.

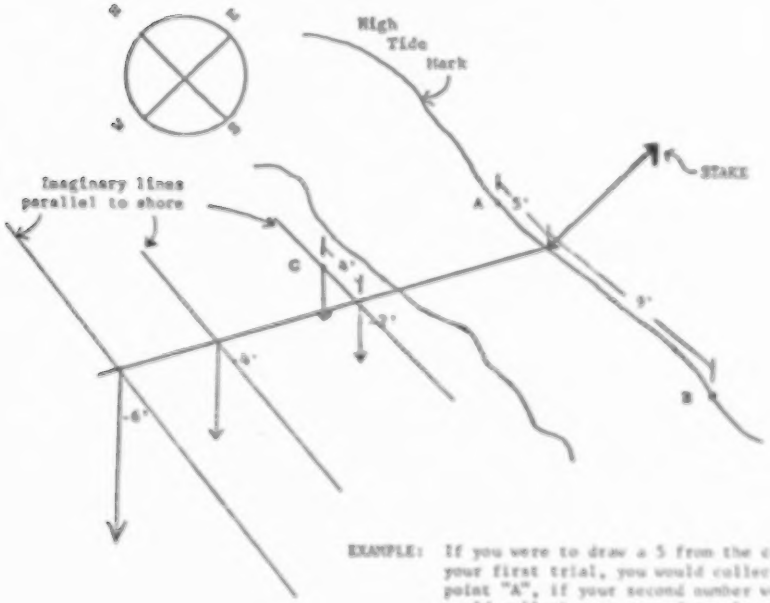
1.5 At each location (elevation level) two samples are to be taken at random to eliminate the possibility of obtaining a biased sample.

bi' ased = Not a typical sample

1.5.1 Prepare a container with ten slips of paper marked 1 to 10.

1.5.2 After you have established the elevation location for sampling in the field, project an imaginary line parallel to the shore line. See Figure 3.

Figure 3. Locating the Sampling Areas at the Station



EXAMPLE: If you were to draw a 5 from the container on your first trial, you would collect a sample at point "A", if your second number were 9, you would collect your second sample at point "B." Drawing again, if the number were 4, collect at point "C", etc.

METHOD: (Population Studies, Continued)

1.5.3 Draw a slip of paper from the container. Measure a distance in feet along the imaginary line parallel to the elevation equal to the number you drew. This will be the exact location where you will take your sample.

1.5.4 Return the slip to the container and collect the sample. You will draw a second time to obtain the exact location to the other side of the line running from shore where the second sample will be taken.

1.5.5 Repeat this procedure to locate the collecting sites at each elevation.

2.0 Collection of Uniform Samples

2.1 There are many commercial devices for removing a uniform sample of mud or sand for studies such as this which may be used. However, it is not only more economical to "build your own," but equally satisfactory.

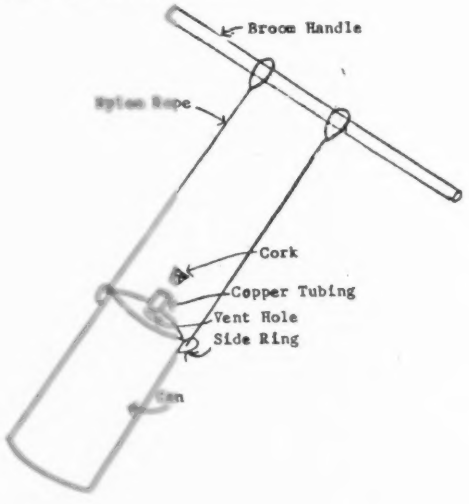
2.2 The following diagram will serve as a guide for constructing a "Bio-Core" sampler.

a) The can should be at least 20 cm long and between 6 to 10 cm diameter.

b) The container should be smooth walled, preferably without ridges around the circumference.

c) It is desirable to sodder a piece of copper tubing to the opening on the top.

Figure 6. Bio-Core Sampler



d) The rings should be riveted or bolted to the side of the can firmly.

METHOD: (Population Studies, Continued)

2.3 Carefully push "Bio-Core" sampler into the ground. Insert cork in the top opening. Place a pole through loops and carefully lift.

2.4 The contents of sampler should be carefully "slipped" into a slightly larger container to be transported back to the laboratory for analysis.

2.5 To prevent decay, keep the sample cool. It may be stored overnight in a refrigerator. Cover with plastic wrap or a plastic bag.

3.0 Separation and Identification of Specimen

3.1 Place the core in a large pyrex baking dish or finger bowl.

3.2 Trim the bottom of the core to a length of 18 cm. (Should the core be crushed or short, be certain to RECORD this on your data sheet.)

3.3 Using forceps, tweezers, needles, etc., carefully and systematically remove all specimens. It is frequently desirable to use an eyedropper and isotonic salt (NaCl) to "flush out" smaller organisms.

i so ton' ic = a sodium chloride (NaCl) solution containing a salt concentration equivalent to that found within the animal. A sample of water from the station will work well.

3.4 Be careful not to overlook small forms or those which blend in with the granular material. When the larger, more obvious organisms have been removed, carefully scan the entire sample with a dissecting microscope.

3.5 Sort out the organisms into similar groups. It is often better to work on live specimens. Organisms may survive if they are kept in a refrigerator. If they die, place them in a 5% formalin solution to prevent decay.

3.6 For identification, use the *Key to the Chordates and Invertebrates of the San Francisco-Delta-Estuary Complex*, developed for project MER. Those organisms not found in the *Key* should be carefully preserved and labeled. You may wish to work with your instructor using other keys to identify the organism.

3.7 It is desirable, when at all possible, to return the living organisms to their environment when you have completed your identification. Make every attempt to do this.

METHOD: (Population Studies, Continued)

4.0 Tabulation of the Numbers of Each Species

4.1 On the Data Sheet, indicate the number of each species collected in the appropriate place.

5.0 Determination of Biomass of Species Present

(Instructions for this part of the research will be treated separately.)

6.0 Calculations of Population Densities in Number /M^2

6.1.0 The sampler you used removed a portion of a square meter. If the area taken by the sampler is calculated, it is a simple procedure to convert your density, $\frac{\text{number of organisms}}{\text{sampler area}}$, to standard units which others will be able to use. Several methods exist; the following is suggested:

6.1.1 Carefully measure the inside diameter of the sampler.

6.1.2 Substitute the appropriate values in the formula,

Area of a circle = πr^2 where

r = 1/2 the diameter and $\pi = \frac{22}{7}$,

to calculate the area of the sampler in cm^2.

6.1.3 The area of M^2 (square meter) in cm^2 equals 10,000 cm^2 or 10^4 cm^2.

cm^2 = square centimeter

6.1.4 This is the portion of the square meter you have sampled

> Example: If the area of the cylinder was previously calculated as 500 cm^2, this would be $\frac{500\ cm^2}{10{,}000\ cm^2}$ = .05th of the area.

6.1.5 To express the density of the population in number of organisms /M^2, we will assume that the sample you have taken is representative of an M^2 at that distance from the shore.

METHOD: (Population Studies, Continued)

6.1.5 (Continued) Therefore, it is necessary to increase the number of organisms (species) found in the sampler, proportionally to portion of the area sampled. To do this, we:

$$\frac{1.00 \text{ Total area}}{\text{Area sampled}}$$

$\frac{1}{.05}$ = Total area is 20 x greater than sample.

6.1.6 The number per M^2 is equal to:

$$\left(\frac{\text{Total area}}{\text{Area sampled}}\right) \times \left(\frac{\text{Number of organisms of a species in A + B samples}}{2}\right)$$

Example: Assuming that there were 5 worms of a species. $\frac{1}{.05} \times (5) =$ 100 worms/M^2 at station Y on a specific day and time at R elevation of the tide.

6.1.7 When you collected your samples in the field, you took two samples at each tide level. Each sample at this same distance from the high water mark is to be considered as part of the *same* population. Therefore, we can average the number of the same species in each sample and use this value in our formula. This will help minimize the chances of having a biased sample for comparison. The formula will then read:

The number of a species per M^2 equals:

$$\left(\frac{\text{Total area}}{\text{Area sampled}}\right) \times \left(\frac{\text{Number of organisms of a species in A + B samples}}{2}\right)$$

Example: Assuming that there were 5 worms of a species in sample A and 3 of the same species in sample B,

$(0.95) \times \frac{5 + 3}{2} = 3.80$ worms/M^2 at station Y on a specific day and time at R elevation of the tide.

METHOD: (Population Studies, Continued)

6.1.8 For each of the populations you have identified, calculate its population density and RECORD this value on the Data Sheet.

DISCUSSION:

The information you have gathered will illustrate some important relationships that exist in marine communities subject to tidal fluctuations. Evolutionary adaptation has resulted in many organisms which are capable of withstanding periods of time out of direct contact with the circulating waters. Such organisms which are present in our waters can be clearly identified from the data you have gathered. Which ones have this ability? What might happen to the various species you have identified if the tidal fluctuations were interfered with as often happens when man changes or modifies tidal conditions? Could this affect other organisms in a food web? What experiments could you design or information could you gather to better understand and predict what might happen to the animal populations in an area if tidal patterns were modified?

Aside from the immediate knowledge you will gain from a single sampling, your data will be of great value to the *Monitoring Program* of Project MER. As the station you have established is sampled monthly, changes in the densities of the populations will be recorded. Some populations may remain fairly constant, others will fluctuate. Very little is known to biologists about these population fluctuations and will be of value in understanding the ecology of our water system. When sufficient data is collected, a base line which will indicate the densities of particular organisms over a yearly period can be established. *As changes in water quality and tidal patterns occur, data will be available, the base line data you have collected, which will enable future students to measure accurately the resulting changes in the ecology of our waters. Such information will enable man to more clearly understand his effect on the environment.*

M E R

Surveying Pelagic Populations with a Beach Seine

INTRODUCTION:

A classic method of surveying populations involves the use of a beach seine. The seine is nothing more than a length of strong mesh material with uniform openings. The upper end has flotation devices attached and the lower edge is weighted. In principle, the net is opened out in the water and is moved toward the shoreline over a smooth bottom. When properly handled, and this takes practice, one can trap most of the organisms in the path of the net. Obviously, many faster organisms will escape, but this method is still used as a basic sampling procedure by biologists.

As part of the Project MER Monitoring Program, monthly assessments of pelagic populations using the seine at the different Field Stations will be necessary. You should practice using the seine before attempting to collect valid data or your results will be misleading.

NOTE: *A note of caution. It is against the Fish and Game Laws to use a beach seine WITHOUT a valid Scientific Collector's Permit. These can be obtained by teachers for use in Project MER through the Project Director in the Contra Costa County Department of Education Office.*

Instruction in the proper use of the beach seine will be introduced at the Point Molate Marine Laboratory. As part of the MER Research Design, a net 4 ft. X 20 ft. with a 1/4 mesh size is required to insure that the conditions for trapping the pelagic forms are as uniform as possible at all Field Stations.

MATERIALS:

Beach seine of 1/4 mesh, 4 ft. by 20 ft. with necessary ropes for pulling through the water.
A 20 ft. rope knotted at 1 ft. intervals for measuring purposes.
Suitable plastic or canvas buckets for sorting out specimen for identification.
Key to the Fishes of the San Francisco Bay-Delta-Estuary Complex, Project MER (in production)

MATERIALS: Continued

COMMON OCEAN FISHES OF THE CALIFORNIA COAST
Phil M. Roedel, State of California Department of Fish and Game, 1953. Fish Bulletin No. 91

A FIELD GUIDE TO SOME COMMON OCEAN SPORT FISHES OF CALIFORNIA
Daniel J. Miller, Dan Gotshall, and Richard Nitsos
Second Revision, April 1965, State of California Department of Fish and Game

FRESHWATER NONGAME FISHES OF CALIFORNIA
J. Bruce Kimsey and Leonard O. Fisk
State of California Department of Fish and Game 1964

OFFSHORE FISHES OF CALIFORNIA
John E. Fitch
State of California Department of Fish and Game 1969

KEYS TO THE FRESHWATER AND ANADROMOUS FISHES OF CALIFORNIA
J. B. Kimsey and Leonard O. Fisk
Reprint from California Fish and Game Quarterly, Volume 46, Number 4, October 1960

Waders - appropriate sizes
Fish measuring board (metric)
2 Float bottles with 4 ft. rope attached to bottom for markers

METHOD:

1. Following the procedure you learned at the Point Molate Marine Laboratory, bring the seine net out into the water. It is best to plan to seine this at slack tide.

2. Prior to beginning to pull the net in, each net puller should release the float so that he will be able to note the exact place where he began to seine.

3. Keeping the distance between the two seiners as constant as possible, bring the net onto the shore as you were instructed.

HANDLING THE SPECIMEN

4. Immediately, those assisting should sort out the organisms. Handle each as little as possible to prevent injury or death. *(Removing the slime on fish by handling enables parasites to more readily parasitize them.)*

METHOD: (Continued)

5. When the organisms have been separated, they should be identified IN THE FIELD. If you have practiced using the Keys, there should not be any need to remove specimens and bring them to the laboratory. If necessary, take pictures of those you cannot identify for future reference. This is certainly a better alternative than returning to the school with them IF you are interested in preserving the populations of organisms you are monitoring.

6. RECORD the total number of each species of fish you catch. Using the Fish Measuring Board, RECORD the length of the Largest and Smallest of each species. Measure from the head to the inside of the tail if it is forked.

7. In the case of the invertebrates, note the species captured and the numbers of each. Average sizes should be RECORDED.

8. If you notice that any of the organisms are gravid this information should be RECORDED.

9. If any of the specimen are damaged, RECORD this information.

DETERMINING THE VOLUME OF WATER SAMPLED

10. It is difficult to determine the exact volume of water sampled but it is possible to make a fairly good estimate by the following procedure.

Using the rope which has been knotted at one ft. intervals, measure the distance between the two float bottles which were released at the time when the seining operation began. RECORD this distance to the nearest 1/2 ft.

11. Measure the depth of the water where you began. RECORD.

12. Using the same rope, measure the distance from the shore to the float bottle. RECORD.

13. If you were to assume that there was no slope up to the shore, you would have passed through a uniform column of water whose volume could be determined simply by using the following formula:

Volume of Water in Column =

$$\text{Distance between Seiners} \times \text{Depth of Water} \times \text{Distance to Shore}$$

METHOD:

13. (Continued)

EXAMPLE:

Assume that: the distance between seiners = 19 ft.
the water depth = 4 ft.
the distance to shore = 200 ft.

Then:

$19\ ft. \times 4\ ft. \times 200\ ft. = 1440\ ft.^3$

14. At this point, you will have to make some judgments. If the slope were uniform, the volume of water you actually sampled would be equal to 1/2 the figure calculated above. Such ideal conditions will be infrequent. More likely, than not, the slope will be gradual for part of the way and then change. You will have to figure out how to handle this problem with the help of the others in the group.

EXAMPLE:

Assuming an even slope, the estimated volume actually sampled equals:

$1440\ ft.^3 \times 1/2 = 720\ ft.^3$

15. Calculate the Estimated Volume of Water Samples in feet.^{3} RECORD.

METHOD: (Continued)

16. Since most of our work will be in metric units, this estimated volume in cubic feet will be converted into metric units. Since 1 cubic ft. equals 0.02832 M^3, one has only to multiply the Estimated Volume of Water Sampled in Cubic Feet by 0.02832 to convert this value into metric units. Calculate the Estimated Volume of Water Sampled in Cubic Meters and RECORD.

EXAMPLE:

Assume that you have estimated that the volume of water sampled equals 720 cubic feet.

Then:

$0.02832 \times 720 = 20.39\ M^3$ Estimated Volume of Water Sampled in Cubic Meters

CALCULATING POPULATION DENSITIES

17. For each of the species captured, and recorded, calculate the population in numbers per cubic meter by dividing the number of each species caught by the Estimated Volume of Water Sampled. RECORD the population density of each species.

EXAMPLE:

Assume that there were 4 Stripped Bass caught in the seine.

Then:

$$\frac{4 \text{ Bass}}{20.39\ M^3 \text{ Water}} = 0.20 \text{ Stripped Bass}/M^3$$

MER

Surveying Populations in a Rocky Intertidal Area

INTRODUCTION:

If you have ever enjoyed looking at life in a tide pool or rocky intertidal area, you are certainly aware that many organisms scurry about quickly and many others are well hidden under rocks. Upon moving a rock there is usually a rush of activity as many of the organisms race for cover in the numerous crevices and openings in the area. If one wanted to determine the exact numbers of the various species in a given area, he would find the task exasperating. Even the best trained and "quickest" of field biologists have difficulties, and at best their counts are estimates. The degree of reliability of their data is largely based on their experience and the care with which they proceed.

There will not be too many rocky areas to be surveyed at the various Field Stations, but where they exist, they should be monitored as part of the research design of Project MER. If you should have the opportunity to survey such an area, do it carefully as this data will be very important in assessing the kinds of organisms present in the San Francisco Bay-Delta-Estuary Complex.

MATERIALS:

A ring or square with an area of approximately 0.25 M^2
Several plastic or canvas buckets
An 8 meter length of rope knotted at 1/2 meter intervals
Keys to the Invertebrates of the San Francisco Bay-Delta-Estuary Complex. James Shettler, Contra Costa County Department of Education, 1971.
Other Keys
Between Pacific Tides, 4th Ed., Revised by Joel W. Hedgpeth, 1968. Ricketts and Calvin

METHOD:

1. In BETWEEN PACIFIC TIDES, you will find a clear description of the zonation which occurs in rocky intertidal areas. Examine the Field Station carefully. Even at low tide it is possible that all zones may not be present, particularly if the rocky area extends into mud or sand flat.

2. Sketch your Field Station carefully at a low tide and measure the distance from a bench mark to the middle of those zones which you can identify. RECORD this information for future reference.

METHOD: (Continued)

3. Drop the ring or square randomly within Zone 1. Do not yield to the temptation to pick an interesting area.

4. Working slowly and carefully so as not to send all the animals scurrying out of the area, attempt to identify the various species present and indicate the approximate numbers of each species within the confinds of the ring.

5. RECORD the species and their approximate numbers on the Data Sheet.

6. Repeat the above procedure at each of the Zones which can be identified at the particular Field Station. RECORD the data.

7. When you return to the laboratory, you can easily calculate the density of the populations present. Since you know the area within the zone you counted, you need only divide the number of a given species by this value to determine the number of the species per square meter. RECORD the density of each species on the data sheets.

EXAMPLE:

Assume that you have determined that the area of the circle or square which you used in Zone 1 was 0.25 M^2, and that you identified 35 specimens of the crab *Hemigrapsis nudis*, you would procede as follows:

$$\frac{35 \text{ specimens}}{0.25\ M^2} = 140 \text{ Hemigrapsis nudis}/M^2$$

MER

Surveying Populations on Pilings

INTRODUCTION:

Pilings form natural homes for many types of organisms. Aside from the frequently observed mussels and barnacles, there are populations of sponges worms, and small crustaceans which live between the larger forms. Observing small "patches" of encrusting forms under a stereoscopic microscope will greet the observer with a fascinating collection of smaller forms of great interest and diversity.

The kinds of organisms found on particular pilings vary considerably depending upon the season and salinity of the waters. As is the case on the rocky intertidal area where different species tend to congregate at different levels or zones, organisms which encrust on pilings tend to congregate in different zones.

As part of the MER Monitoring Program, selected pilings at the different Field Stations will be monitored to record changes which occur in the populations of organisms at the different Field Stations and at the different zones at these stations.

MATERIALS:

Spatula or sharp knife for removing a sample of encrusted organisms
Collection jar (wide mouth "skippy peanut butter size is fine)
Stereoscopic Microscope
Ample small containers and vials
Gallon jug
Dissecting needles
Droppers
Concentrated formaldehyde
10% formaldehyde or 70% alcohol
Metric graph paper, 10 mm to the cm, linear
Key to the Invertebrates of the San Francisco Bay - Delta - Estuary Complex, James Shettler, Contra Costa County Department of Education, 1971

METHOD:

1. Examine the piling which is to be periodically sampled at the lowest tide. After having read the first section of *Between Pacific Tides*, sketch the piling and identify the zones which occur. With the use of a metric tape, measure the position of each of the identifiable zones and RECORD this data for future reference.

METHOD: (Continued)

2. You will want to remove a small sample, approximately 25 cm^2 (5 cm x 5 cm) from each of the zones for analysis. The object is to select your sample within each zone <u>at random</u>. Discuss this problem with your instructor before removing any specimen. There are many ways to do this and you should decide on a method and RECORD this on your Data Sheet.

3. Remove a random sample of encrusting organisms from each of the zones present, trying to keep the sample intact.

4. Place the intact sample in a numbered collection jar with ample water.

5. Collect about a gallon of water which you will reserve for later use.

6. It is best to begin with living material. If you can get the sample back to the lab alive, it will hold for about 24 hours in a refrigerator with the top loose. If this is not possible, you should add sufficient formaldehyde so that the water in the container will be at approximately 10% dilution.

7. <u>Determining the size of the sample</u>. Since the sample is most likely irregular in shape, the following procedure is suggested:

   a. Carefully center the sample in a clean petri dish
   b. Place the dish on metric graph paper (10 mm to the cm, linear)
   c. On a second piece of graph paper, copy the outline of the specimen.
   d. When you have completed, count the number of squares to determine the area of the sample. RECORD the area of each sample in cm^2.

Figure 1
Diagram of sample on graph paper

Figure 2
Diagram of area covered by sample

METHOD: (Continued)

EXAMPLE:

Assume that *Figure 1* represents an outline of the sample you are working with.

Each major square on the graph paper = 1 cm^2
Each small square measures 0.2 cm by 0.2 cm. Therefore, the area contained in the small square = 0.04 cm^2.

In this example, the number of large squares covered by the sample = 11 and the number of small squares = 190

Therefore:

Number of 1 cm squares (11)	$= 11.0\ cm^2$
Number of 0.04 cm squares (190) X ($0.04 cm^2$)	$= 7.6\ cm^2$
Area of the sample in cm^2	$18.6\ cm^2$

8. To identify the organisms, begin teasing the sample apart, sorting out the organisms into similar kinds and placing them into small containers. Use the water you collected for diluting.

9. Preserve the organisms in 10% formaldehyde or 70% alcohol.

10. When you have completed the sorting, identify each using the *Key to the Invertebrates of the San Francisco Bay - Delta - Estuary Complex*, and other sources available. A stereoscopic miscroscope will be useful for this purpose.

11. On the Data Sheet, RECORD the species identified and the numbers of each present in the sample.

12. The standard method for reporting the density of organisms for this monitoring program will be in numbers per square decimeter. Since a decimeter is equal to 10 centimeters, a square decimeter (dm^2) equals 100 square centimeters. Therefore, all you have to do is divide the number of organisms of a given species counted by the sample size (which is the number per cm^2 and multiply that value by 100 to convert to number per dm^2. RECORD this data.

$$\frac{\text{Number of a species in a sample}}{\text{Size of the sample in } cm^2} \times \frac{100\ cm^2}{dm^2} = \frac{\text{Number of a species}}{dm^2}$$

METHOD: (Continued)

EXAMPLE:

Assume that you had counted 7 barnacles of the Genus *Balanus*. Your calculations for expressing the population density in number of organisms/dm^2, using the sample previously calculated would be:

$$\frac{7 \text{ Balanus}}{18.6 \text{ cm}^2} \quad \frac{100 \text{ dm}^2}{\text{cm}^2} = 37.6 \text{ Balanus spp./dm}^2$$

M E R

MONITORING PROGRAM

CHAPTER III

TECHNIQUES FOR STUDYING BACTERIAL POLUTION

in the

SAN FRANCISCO BAY - DELTA - ESTUARY COMPLEX

MER

Analysis of Water for Bacterial Pollution

Prepared by Marcia Sakanashi
(Senior, Kennedy High School, Richmond)

BACKGROUND:

Bacteria, as part of the natural food web in any body of water, play an important role as the "decomposers." In such a role they break down organic material, releasing nutrients necessary for the growth of plants. Parasitic bacteria, also found in waters, serve to remove weakened organisms, and in cases of overcrowding of a particular species, can cause epidemics which tend to keep these populations in check. To a large extent the number and kind of bacteria present depend upon the source of the water, its temperature, and the types and concentrations of dissolved minerals present. Artesian wells and springs have a lower bacteria count than shallow ponds and streams. Sewage and waters heavily polluted with organic materials contain millions of bacteria per milliliter. These bacteria may be introduced into waters effluent with sewage and continue to grow on the rich nutriant medium of which sewage is composed. Coliforms, always found in the intestinal tract of man and animals, are expended by billions in feces and find their way into the water supply with sewage. Though coliforms are not harmful themselves, their presence is a sure indicator of sewage pollution and the almost certain presence of other bacteria that are harmful. The accepted method of determining whether or not a potable water has become polluted is to determine whether coliform bacteria are present in excessive amounts.

In densely populated areas water pollution by sewage is an ever present hazard. Several serious diseases can be traced to polluted drinking water. Among them, typhoid fever and a group of intestinal disorders generally lumped under the name of "disentary." The actual disease causing organisms, such as *Salmonella typhosa*, may be extremely hard to detect. Instead of testing for these health bacteria directly, authorities routinely check for the presence of coliform bacteria, which we have already mentioned as an indicator of water pollution. Coliforms, only always present in sewage, are much harder to kill off in treatment plants than the actual disease producers. If they are not present in the test sample of waste water, we can be reasonably sure that no other bacteria due to sewage pollution are there either.

Today, millions of dollars are being spent in programs to clean up the rivers, lakes and smaller streams in the United States. Aside from the fact that sewage pollution is a disease hazard, it is of broader ecological importance

BACKGROUND: (Continued)

in that it may bring abnormally large numbers of bacteria to an otherwise normally balanced water system. Since some bacteria use significant amounts of oxygen in their life process, they remove it from the water and it becomes unavailable for fish and other aquatic life. Periodic fish kills can often be traced to sudden oxygen depletion from such a cause. Many streams in this country that were once alive with fish are now nothing but festering cesspools.

There are only two methods currently available for the detection of coliform bacteria which serve as an index of pollution in water. The traditional method is the "Multiple-Tube Fermentation Method," which has been employed for many years. The newer "Membrane Method," has resulted from recent technological advances which permit the production of filters with uniform openings permitting the separation of bacteria from water.

The "Multiple-Tube Fermentation Method," involves innoculating a series of lactose broth tubes with water samples. If coliform bacteria are present, a gas is produced which is collected in inverted vials contained within test tubes. The degree of bacterial contamination is derived by analyzing the amount of gas produced under stated conditions.

In the "Membrane Method," a sample of water is passed through a filter to separate the bacteria. When the bacteria collected are grown in petri dishes on a special media, the coliform group will produce an easily recognizable colony.

Both methods are outlined in *Standard Methods for the Examination of Water and Wastewater*. As part of the MER Monitoring Program, the analysis of waters for Total Coliform is included. The method included in this Handbook is the "Membrane Method."

MER

TOTAL COLIFORM ANALYSIS

MILLIPORE MEMBRANE METHOD

Prepared by Marcia Sakanashi
(Senior, Kennedy High School, Richmond)

MATERIALS:

Water sample(s)
Millipore filtration unit with hand pump
Petri dishes, 47mm
Millipore filters with absorbent pads, HAWG 47 mm, 0.45μ , sterile
Forceps, blunt
Household bleach (Sodium hypochlorite ≈ 5-6%
70% Alcohol for sterilizing
Media - Ampule containing 2 ml MF-Endo Medium (pink)
Ampule breaker
Sterile pipette, 1 ml in 1/10
Sterile pipette, 50 ml OR 100 ml graduated cylinder
Sterile dechlorinated water
Incubator
Binocular scope, hand lens OR Colony counter

CULTURE MEDIUM is the "soil" for growing microorganisms, and contains the food nutrients required for growth. In general, all microorganisms require carbon, nitrogen, and various other growth factors and vitamins. A typical "simple" nutrient medium might consist of some sugar (such as dextrose) to supply the carbon, protein or protein-breakdown products for the nitrogen, and a water extract of meat for the various other growth factors needed. Dissolved in water, this medium is known as a liquid medium. It can be adjusted to a neutral point--not too acid or too alkaline--suitable for growth of most bacteria, and can be solidified if desired by the addition of gelatin or an extract of seaweed known as agar.

MF-ENDO MEDIUM, pink as cherry blossoms, commemorates a doctor in Tokyo named Endo, who developed it in simpler form. This is the medium used to verify the presence of coliform bacteria, and how it does so is a perfect example of microbiological detective work. Years ago, researchers learned that coliform bacteria have a unique ability to break down a complex "sugar" called lactose, forming a definite sequence of simpler substances. Some of these, showing up about 36 hours after the process starts, are called aldehydes.

The MF-ENDO MEDIUM contains lactose and other nutrients, and also a stain--basic fuchsin--which is normally deep red but has been made pale pink by the addition of sodium sulfite. The first action of bacteria...in fact, of the air itself...is to partly reverse the effect of the sulfite, making the whole surface of the medium red. Most colonies growing there also become red, but with nothing to distinguish one species from another until the aldehydes are formed. When this happens, the unchanged fuchsin-sulfite complex (plenty of it is still around) attaches itself to the aldehyde molecules and forms a shiny green coating. Because no microorganisms make aldehydes out of lactose except members of the coliform family, "green sheen" colonies are coliform bacteria, and the sample is proved guilty of pollution by sewage.

MILLIPORE FILTERS. These are special "membrane" filters containing billions of microscopically uniform small holes or pores per square inch.

If a liquid sample is passed through a filter whose pores are smaller than the microorganisms in the sample, all the microorganisms will stay on top of the filter. Since none of them are left in (the filtrate) it is sterile.

Filters are normally supplied sterile in resealable envelopes. Each envelope contains 10 gridded filters, and 10 pads which look like blotter paper. These are called absorbent pads and are used as the name implies, to absorb and hold the liquid nutrient material used to nourish the growth of bacteria. If the filters you are using are supplied in such a sterile pack, remove one carefully, using sterile forceps, and reseal the envelope. The blue wax paper discs in the envelope are merely part of the packaging and should be discharged.

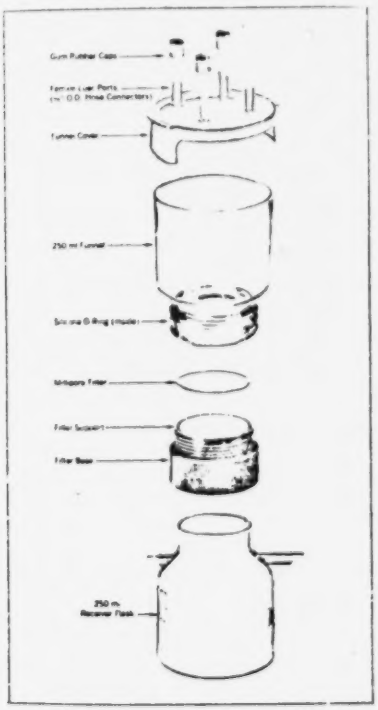


Figure 1

The STERIFIL FILTRATION APPARATUS is the central piece of equipment used in these experiments. Its component parts are shown in Figure 1. Before each experiment, the funnel and filter base must be sterilized. The receiver flask does <u>not</u> require sterilization. This is because we don't have to be aseptic about what passes through the filter, but only about the sample that is on top of the filter, and anything that touches that sample.

The Sterifil funnel cover should be used when you want to prevent airborn spores and microorganisms from falling into the Sterifil funnel while the sample is being filtered. It is unnecessary in this procedure because coliforms are not likely to be found in the air.

The VACUUM SYSTEM, used to operate the filtration unit, consists of a plastic syringe, a length of rubber tubing, and a two-way valve. One end of the tubing carries a small nylon adapter. Slip the other end tightly over the side vent of the valve, and attach the valve to the syringe (Figure 2.) Insert the nylon adapter into one side arm of the Sterifil receiver flask (Figure 3), and close the other side arm with a gum rubber cap.

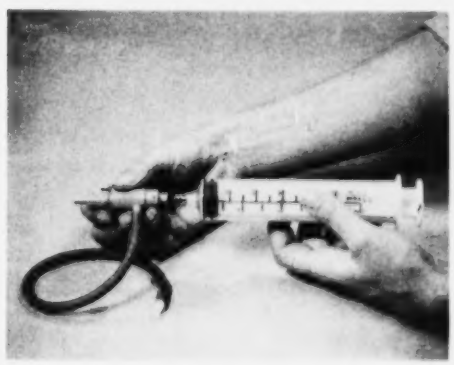


Figure 2

When the syringe plunger is worked (Figure 4), air will be drawn out of the receiver flask and vented through the valve outlet. This will quickly create sufficient vacuum inside the receiver flask to start and maintain the filtering process, perhaps with an occasional "boost" from the syringe.

Figure 3

Figure 4

PETRI DISHES are named for Julius Petri, a German bacteriologist who first used thin glass dishes with covers to contain culture media on which microorganisms were being grown. The petri dishes to be used are made of transparent plastic, with close-fitting covers that help keep the media from drying out. They are sterile as supplied, and cannot be boiled without injuring the plastic. However, they can be resterilized by a special soaking technique described below:

1. Using forceps, carefully remove the petri dish covers and then put the covers and dishes (with cultures) into a large beaker or pan containing liquid household bleach (straight from the bottle.)

2. After 10 minutes, remove the petri dishes using tongs or a rubber glove and rinse them well under running water. The wet pads and filters should be put into a plastic bag and discarded.

3. Immerse the petri dishes and covers in a solution of 70 percent alcohol for 10 minutes (you can use rubbing alcohol available at any drug store or supermarket.)

4. After 10 minutes, remove the petri dishes and covers and stack them on a clean surface as shown in Figure 5. Then assemble the dishes and covers. They are now ready for use.

MILLIPORE MEMBRANE METHOD - (Continued)

ASEPTIC TECHNIQUE is the name we give to the key factor in doing microbiological things the right way. "Aseptic" means free from disease-causing microorganisms, but is generally broadened to mean free from any microorganisms that are unwelcome at the particular place and time. Although the great majority of microorganisms are not pathogenic, a good microbiologist treats all cultures as if they were. The techniques he uses have been carefully worked out to exclude the contamination from "outside" any experiment, to make sure that all media flasks and other equipment used are sterile, and to prevent the escape of microorganisms from the experiment into the outside world.

Figure 5

Such techniques not only safeguard the microbiologist and his fellow workers, but also ensure that the cultures he is working with do not get contaminated by bacteria from the environment. The equipment provided for our own experiments has been specifically planned with aseptic techniques in mind.

METHOD:

1. Collect a sample of salt water at the Field Station using a sterilized container that can be capped shut during transportation.

2. Prepare the following:

   a. Sterifil apparatus, with type HAWG filter.

      (1) Sterilize the funnel and the filter base by immersing both in rapidly boiling water for 3 minutes. After boiling, lift the funnel and filter base out of the water with tongs and place them on a clean sheet of wrapping paper. The funnel should rest upside-down on its larger rim, while the filter base rests with the filter support area up. Do not wipe these units, and do not touch the inside of the funnel or the top of the filter support with your fingers. Allow the pieces to drain for a moment or two, then press down the filter base firmly over the top of the receiver flask.

      NOTE: Because the plastic filter funnel has a higher expansion coefficient rate of expansion when heated than the red silicone rubber O-ring, the O-ring may tend to fall out of its groove during boiling. If this happens, place the O-ring back in the groove using sterile forceps. Then screw the blue filter base into the funnel until the O-ring seats properly.

(2) The test filter should now be installed. (This is 47 millimeters in diameter, and called a Type HAWG...which is catalog code meaning 0.45 pore size, white, with a grid-marked top surface.)

First sterilize the forceps by dipping the tips in alcohol and while still coated with a thin layer of alcohol passing it quickly through the flame of the alcohol burner. This will ignite the residual coating of alcohol on the forceps' blades thus sterilizing it. Let the forceps cool briefly, then use it to lift a single filter out of the box. Put the cover back on the box immediately, and with the forceps, place the filter on top of the filter base, centering it accurately. (Figure 6)

Figure 6

NOTE: Millipore filters will "flash burn" if accidentally ignited. Let the forceps cool for a few seconds after flaming, before you use them to pick up the filter, which otherwise might be scorched.

If there is any residual moisture on the filter base, it will wet the filter slightly, but this does no harm. Complete the assembly by screwing the funnel down over the filter base (Figure 7.) Be careful not to use too much force, or the filter itself might get torn and rendered useless.

Figure 7

b. One petri dish and pad, with MF-ENDO medium (pink.)

(1) To open a petri dish, insert a forceps blade between the sections and pry with a twisting motion (Figure 8.) Be careful not to touch the inside surface of either section. Using flamed forceps, a 47 millimeter absorbent pad can now be placed in the bottom section (Figure 9) to receive the selected culture medium. The face with raised lettering should be treated as the bottom, keeping the clear face as the top for easier observation of growth.

Figure 8

Figure 9

(2) The media used are supplied in the form of sealed glass ampoules. Each ampoule contains the exact amount (2 ml) of sterile liquid medium needed to saturate a single absorbent pad. To use this form of medium, select an ampoule of the proper medium and holding as is shown in Figure 10, pass it quickly through the alcohol flame while turning it so that all sides of the indented "neck" are sterilized. Then, insert the ampoule into the ampoule breaker, and holding the bottom of the ampoule, squeeze the sides of the breaker together to snap the neck (Figure 11.) Not much force is needed. Pour the contents of the ampoule evenly over the absorbent pad in the petri dish, making sure that the entire pad is saturated (Figure 12.) If (as sometimes happens) a small shred of glass from the broken ampoule gets onto the pad, simple lift it off with flamed forceps, then replace the petri dish cover loosely until sample filtration has been completed.

Figure 10

Figure 11

Figure 12

3. Pipette 50 ml of sterile dechlorinated water into the Sterifil funnel.

4. Pipette an 0.5 ml aliquot of the Bay sample water into the Sterifil funnel, and swirl the funnel to mix the sample with the sterile dilution water. (The size of the aliquot will vary with the contamination level of the water being sampled. Until this determination is done, start with an aliquot size of 0.5 ml.)

5. Filter the diluted sample. Pipette another 50 ml of sterile dechlorinated water into funnel and filter through. This is to prevent the bacteria from clumping or becoming encrusted in salt, since the sample is taken from salt water. After filtration of the test sample, release the vacuum in the system by removing the vacuum pump tubing from the side-arm of the Sterifil receiver flask. Unscrew the funnel, and using flame-sterilized forceps, lift the filter from the Sterifil base and place it gridded-side up on the saturated absorbent pad in the petri dish. Carefully line up the filter with one edge of the petri dish and then set it down with a slight rolling motion (Figure 13), so that it is evenly centered. Replace the cover, invert the petri dish, and set it aside in a warm (not <u>hot</u>) place to "incubate" for the required length of time.

6. Allow the culture to incubate for 48 hours at normal room temperature, or 24 hours at 37°C if you have an incubator. At the end of that time, remove the test filter with flamed forceps and allow it to dry on a clean blotter for 1/2 hour. (Therefore, do not worry if before, during, or after incubation, there is an excess of media in the petri dish.)

Figure 13

7. With a hand magnifier, or colony counter, scan the surface of the filter for colonies having a shiny, greenish surface. Count the total number of these "green sheen" colonies appearing on the filter. This is the number of coliform bacteria present in the 1/2 ml sample of untreated water. Multiply this by 2 to find the number that would be present in one ml of the same water. If in the end result a 1/2 ml sample is too large, use 1/4 ml of the sample and multiply by four.

8. CLEANING UP after experiments is an <u>essential</u> part of good laboratory practice.

   Wash the Sterifil apparatus, syringe. Disassemble the Sterifil and syringe, to clean all parts with warm water and a mild detergent. In disassembling the Sterifil unit, you may find that the blue filter base is so tightly seated on the receiver flask that it cannot be easily removed. This extremely tight fit is caused by the vacuum that was created during filtration. When this happens, replace the funnel portion back on the filter base, grasp the funnel in one hand and the receiver flask in the other, and rock the two parts in opposite directions until the filter base is released. After washing, rinse in clear water, allow to air dry, reassemble the units that were taken apart, and repack everything in the equipment case.

9. Finally, and most important, WASH YOUR HANDS.

APPENDIX I

MER
FIELD DATA SHEET

Date _____ Station _____

Carbon Dioxide
Titrametric Determination (Delta)

TITRATION DATA:

SAMPLE #1 (Surface Water) *(Sample #1)*

Trial 1a

Drops ____ X 5 = _____ppm CO_2

Trial 1b

Drops ____ X 5 = _____ppm CO_2

Average for Surface Water

Trial 1a + Trial 1b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm CO_2

SAMPLE #2 (1 Meter Water) *(Sample #2)*

Trial 2a

Drops ____ X 5 = _____ppm CO_2

Trial 2b

Drops ____ X 5 = _____ppm CO_2

Average for 1 Meter Water

Trial 2a + Trial 2b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm CO_2

MER
FIELD DATA SHEET

Date _____ Station _____

Carbon Dioxide
Titrametric Determination (Delta)

Continued

SAMPLE #3 (Bottom Water) *(Sample #3)*

Trial 3a

Drops ____ X 5 = _____ppm CO_2

Trial 3b

Drops ____ X 5 = _____ppm CO_2

Average for Bottom Water

Trial 3a + Trial 3b ÷ 2 =

___ppm + ___ppm ÷ = ___ppm CO_2

Investigator _____
School _____
Instructor _____

MER
FIELD DATA SHEET

Date _____ Station _____

La Motte Carbon Dioxide Test

TITRATION DATA:

SAMPLE #1 (Surface Water) *(Sample #1)*

Trial 1a

Major divisions ___ X 2.5 = ___
+ # Minor divisions ___ X 0.5 = ___
Dissolved CO_2 in ppm = ___

Trial 1b

Major divisions ___ X 2.5 = ___
+ # Minor divisions ___ X 0.5 = ___
Dissolved CO_2 in ppm = ___

Average for Surface Water

Trial 1a + Trial 1b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm CO_2

SAMPLE #2 (1 Meter Water) *(Sample #2)*

Trial 2a

Major divisions ___ X 2.5 = ___
+ # Minor divisions ___ X 0.5 = ___
Dissolved CO_2 in ppm = ___

Trial 2b

Major divisions ___ X 2.5 = ___
+ # Minor divisions ___ X 0.5 = ___
Dissolved CO_2 in ppm = ___

Average for 1 Meter Water

Trial 2a + Trial 2b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm CO_2

M E R
FIELD DATA SHEET

Date _____ Station _____

La Motte Carbon Dioxide Test

Continued

SAMPLE #3 (Bottom Water) (Sample #3)

Trial 3a

Major divisions ___ X 2.5 = ___
+ # Minor divisions ___ X 0.5 = ___
Dissolved CO_2 in ppm = ___

Trial 3b

Major divisions ___ X 2.5 = ___
+ # Minor divisions ___ X 0.5 = ___
Dissolved CO_2 in ppm = ___

Average for Bottom Water

Trial 3a + Trial 3b ÷ 2 =
___ppm + ___ppm ÷ 2 = ___ppm CO_2

Investigator _____
School _____
Instructor _____

M E R
FIELD DATA SHEET

Date _____ Station _____

Oxygen Determination
Winkler Titration Method (R-S Modified)

TITRATION DATA:

SAMPLE #1 (Surface Water) (Sample #1)

Surface water temperature in °C = ___°C

Trial 1a

Vol. thio. X Norm. X 40 =

___ml X ___N X 40 = ___ppm O_2

Trial 1b

Vol. thio. X Norm. X 40 =

___ml X ___N X 40 = ___ppm O_2

Average for Surface Water

Trial 1a + Trial 1b ÷ 2 =
___ppm + ___ppm ÷ 2 = ___ppm O_2

SAMPLE #2 (1 Meter Water) (Sample #2)

1 Meter water temperature in °C = ___°C

Trial 2a

Vol. Thio. X Norm. X 40 =

___ml X ___N X 40 = ___ppm O_2

Trial 2b

Vol. thio. X Norm. X 40 =

___ml X ___N X 40 = ___ppm O_2

Average for 1 Meter Water

Trial 2a + Trial 2b ÷ 2 =
___ppm + ___ppm ÷ 2 = ___ppm O_2

M E R
FIELD DATA SHEET

Date _____ Station _____

Oxygen Determination
Winkler Titration Method (R-S Modified)

Continued

SAMPLE #3 (Bottom Water) (Sample #3)

Bottom water temperature in °C = ___°C

Trial 3a

Vol. thio. X Norm. X 40 =

___ml X ___N X 40 = ___ppm O_2

Trial 3b

Vol. thio. X Norm. X 40 =

___ml X ___N X 40 = ___ppm O_2

Average for Bottom Water

Trial 3a + Trial 3b ÷ 2 =
___ppm + ___ppm ÷ 2 = ___ppm O_2

Investigator _____
School _____
Instructor _____

M E R
FIELD DATA SHEET

Date _____ Station _____

Hach Titration Method
for Dissolved Oxygen
Determination (Lab)

TITRATION DATA:

SAMPLE #1 (Surface Water) (Sample #1)

Surface water temperature in ^{o}C = ___ ^{o}C

Trial 1a

___ml PAO = ___ppm O_2

Trial 1b

___ml PAO = ___ppm O_2

Average for Surface Water

Trial 1a + Trial 1b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm O_2

SAMPLE #2 (1 Meter Water) (Sample #2)

1 Meter water temperature in ^{o}C ___ ^{o}C

Trial 2a

___ml PAO = ___ppm O_2

Trial 2b

___ml PAO = ___ppm O_2

Average for 1 Meter Water

Trial 2a + Trial 2b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm O_2

Hach Titration Method
for Dissolved Oxygen
Determination (Lab)

Continued

SAMPLE #3 (Bottom Water) (Sample #3)

Bottom water temperature in ^{o}C ___ ^{o}C

Trial 3a

___ml PAO = ___ppm O_2

Trial 3b

___ml PAO = ___ppm O_2

Average for Bottom Water

Trial 3a + Trial 3b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm O_2

Investigator _____
School _____
Instructor _____

M E R
FIELD DATA SHEET

Date _____ Station _____

La Motte Titration Method
for Dissolved Oxygen
Determination

TITRATION DATA:

SAMPLE #1 (Surface Water) (Sample #1)

Surface water temperature in ^{o}C =

Trial 1a

Major divisions ___ X 0.20 = ___
+ # Minor divisions ___ X 0.04 = ___
Dissolved O_2 in ppm = ___

Trial 1b

Major divisions ___ X 0.20 = ___
+ # Minor divisions ___ X 0.04 = ___
Dissolved O_2 in ppm = ___

Average for Surface Water

Trial 1a + Trial 1b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm O_2

SAMPLE #2 (1 Meter Water) (Sample #2)

1 Meter water temperature in ^{o}C = ___ ^{o}C

Trial 2a

Major divisions ___ X 0.20 = ___
+ # Minor divisions ___ X 0.04 = ___
Dissolved O_2 in ppm = ___

Trial 2b

Major divisions ___ X 0.20 = ___
+ # Minor divisions ___ X 0.04 = ___
Dissolved O_2 in ppm

La Motte Titration Method
for Dissolved Oxygen
Determination

Continued

Average for 1 Meter Water

Trial 2a + Trial 2b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm O_2

SAMPLE #3 (Bottom Water) (Sample #3)

Bottom water temperature in ^oC =

Trial 3a

Major divisions ___ X 0.20 = ___
+ # Minor divisions ___ X 0.04 = ___

Dissolved O_2 in ppm = ___

Trial 3b

Major divisions ___ X 0.20 = ___
+ # Minor divisions ___ X 0.04 = ___

Dissolved O_2 in ppm = ___

Average for Bottom Water

Trial 3a + Trial 3b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm O_2

Investigator _____
School _____
Instructor _____

M E R
FIELD DATA SHEET

Date _____ Station _____

Dissolved Oxygen
(Delta Meter Method)

CHECK METER WITH INSTRUCTOR BEFORE USING

TEST: DATA:

SAMPLE #1 (Surface Water) (Sample #1)

a. Water temp. when testing=___^oC
b. Dissolved O_2 reading=___ppm
c. mg/l of O_2 obtained from the Nonograph=___(Step 10a)
d. O_2 sol. in distilled water at the test temp. = ___(Step 10b)
e. Value 10a___
Value 10b___ X METER RDG.___= ___mg/l O_2

SAMPLE #2 (1 Meter) Bottle No. ___ (Sample #2)

a. Water temp. when testing=___^oC
b. Dissolved O_2 reading=___ppm
c. mg/l of O_2 obtained from the Nonograph=___(Step 10a)
d. O_2 sol. in distilled water at the test temp. = ___(Step 10b)
e. Value 10a___
Value 10b___ X METER RDG.___= ___mg/l O_2

SAMPLE #3 (1/2 M From Bottom) Bottle No.___ (Sample #3)

a. Water temp. when testing=___^oC
b. Dissolved O_2 reading=___ppm
c. mg/l of O_2 obtained from the Nonograph=___(Step 10a)
d. O_2 sol. in distilled water at the test temp. = ___(Step 10b)
e. Value 10a___
Value 10b___ X METER RDG.___= ___mg/l O_2

Investigator _____
School _____
Instructor _____

M E R
FIELD DATA SHEET

Date _____ Station _____

Meter Method For
Determining pH

METER DATA:

SAMPLE #1 (Surface Water) (Sample #1)

pH Reading on meter =
Temperature of water in ^oC = ___
Adjustment Figure from pH CORRECTION GRAPH - ___

pH Meter reading + Adj. Figure =

_____ + _____ ___pH

SAMPLE #2 (1 Meter Water) (Sample #2)

pH Reading on meter =
Temperature of water in ^oC = ___
Adjustment Figure from pH CORRECTION GRAPH - ___

pH Meter reading + Adj. Figure =

_____ + _____ ___pH

SAMPLE #3 (Bottom Water) (Sample #3)

pH Reading on Meter =
Temperature of water in ^oC = ___
Adjustment Figure from pH CORRECTION GRAPH - ___

pH Meter reading + Adj. Figure =

_____ _____ ___pH

Investigator _____
School _____
Instructor _____

M E R
FIELD DATA SHEET

Date _____ Station _____

pH Paper Method for
Determining the
pH of Water

DATA

SAMPLE #1 (Surface Water) (Sample #1)

"Brand" of pH paper used = _____
Range of paper used = _____

pH value obtained on the pH paper = _____pH

SAMPLE #2 (1 Meter Water) (Sample #2)

"Brand" of pH paper used = _____
Range of paper used = _____

pH value obtained on the pH paper = _____pH

SAMPLE #3 (Bottom Water) (Sample #3)

"Brand" of pH paper used = _____
Range of paper used = _____

pH value obtained on the pH paper = _____pH

Investigator _____
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Instructor _____

M E R
FIELD DATA SHEET

Date _____ Station _____

Determining the Chloride Ion
Concentration and Salinity
of Water - MOHR Method

TITRATION DATA

SAMPLE #1 (Surface Water) (Sample #1)

Trial 1a

Normality of $AgNO_3$
ml of $AgNO_3$

$$\frac{(__ \text{ml } AgNO_3)(__ {}^{N}AgNO_3)(35.460)}{__ \text{ml (Sample Vol.)}} =$$

_____ Chlorinity in $^{o}/_{oo}$

Trial 1b

Normality of $AgNO_3$ = _____ N
ml of $AgNO_3$ titrated = _____ ml

$$\frac{(__ \text{ml } AgNO_3)(__ {}^{N}AgNO_3)(35.460)}{__ \text{ml (Sample Vol.)}} =$$

_____ Chlorinity in $^{o}/_{oo}$

Average Chlorinity for Surface

Trial 1a + Trial 1b ÷ 2 =

___ $^{o}/_{oo}$ + ___ $^{o}/_{oo}$ ÷ 2 = ___ $^{o}/_{oo}$ Chlorinity

Salinity in $^{o}/_{oo}$ =

(___ $^{o}/_{oo}$ Adv. Cl) (1.805) + 0.03 = ___ $^{o}/_{oo}$ Salinity

Determining the Chloride Ion
Concentration and Salinity
of Water - MOHR Method

Continued

SAMPLE #2 (1 Meter Water) (Sample #2)

Trial 2a

Normality of $AgNO_3$
ml of $AgNO_3$

$$\frac{(__ \text{ml } AgNO_3)(__ {}^{N}AgNO_3)(35.460)}{__ \text{ml (Sample Vol.)}} =$$

_____ Chlorinity in $^{o}/_{oo}$

Trial 2b

Normality of $AgNO_3$ = _____ N
ml of $AgNO_3$ titrated = _____ ml

$$\frac{(__ \text{ml } AgNO_3)(__ {}^{N}AgNO_3)(35.460)}{__ \text{ml (Sample Vol.)}} =$$

_____ Chlorinity in $^{o}/_{oo}$

Average Chlorinity for 1 Meter Water

Trial 2a + Trial 2b ÷ 2 =

___ $^{o}/_{oo}$ + ___ $^{o}/_{oo}$ ÷ 2 = ___ $^{o}/_{oo}$ Chlorinity

Salinity in $^{o}/_{oo}$ =

(___ $^{o}/_{oo}$ Adv. Cl) (1.805) + 0.03 = ___ $^{o}/_{oo}$ Salinity

MER
FIELD DATA SHEET

Date _____ Station _____

Determining the Chloride Ion Concentration and Salinity of Water - MOHR Method

TITRATION DATA

SAMPLE #3 (Bottom Water) (Sample #3)

Trial 3a

Normality of $AgNO_3$
ml of $AgNO_3$

$$\frac{(__ml\ AgNO_3)\ (__^{N}AgNO_3)\ (35.460)}{__ml\ (Sample\ Vol.)} =$$

_____ Chlorinity in $^{o}/_{oo}$

Trial 3b

Normality of $AgNO_3$ = _____ N
ml of $AgNO_3$ titrated = _____ ml

$$\frac{(__ml\ AgNO_3)\ (__^{N}AgNO_3)\ (35.460)}{__ml\ (Sample\ Vol.)} =$$

_____ Chlorinity in $^{o}/_{oo}$

Average Chlorinity for Bottom Water

Trial 3a + Trial 3b ÷ 2 =

___ $^{o}/_{oo}$ + ___ $^{o}/_{oo}$ ÷ 2 = ___ $^{o}/_{oo}$ Chlorinity

Salinity in $^{o}/_{oo}$ =

(___ $^{o}/_{oo}$ Adv. Cl) (1.805) + 0.03 = ___ $^{o}/_{oo}$ Salinity

Concentration and Salinity of Water
MOHR Method

Continued

Investigator _____
School _____
Instructor _____

MER
FIELD DATA SHEET

Date _____ Station _____

Chloride Determination
Titrametric Test (Delta)

TITRATION DATA

SAMPLE #1 (Surface Water) (Sample #1)

Trial 1a

Drops ___ X 10 = ___ ppm Cl^-

Trial 1b

Drops ___ X 10 = ___ ppm Cl

Average Cl^- for Surface Water

Trial 1a + Trial 1b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm Cl^-

SAMPLE #2 (1 Meter Water) (Sample #2)

Trial 2a

Drops ___ X 10 = ___ ppm Cl^-

Trial 2b

Drops ___ X 10 = ___ ppm Cl

Average Cl^- for 1 Meter Water

Trial 2a + Trial 2b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm Cl^-

Chloride Determination
Titrametric Test (Delta)

Continued

SAMPLE #3 (Bottom Water) (Sample #3)

Trial 3a

Drops ___ X 10 = ___ ppm Cl^-

Trial 3b

Drops ___ X 10 = ___ ppm Cl

Average Cl^- for Bottom Water

Trial 3 a + Trial 3b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm Cl^-

Investigator _____
School _____
Instructor _____

M E R
FIELD DATA SHEET

Date _____ Station _____

La Motte Titration Method
for Salinity

TITRATION DATA

SAMPLE #1 (Surface Water) (Sample #1)

Trial 1a

Major divisions ___ X 1.0 = ___
+ # Minor divisions ___ X 0.2 = ___
Salinity in °/oo = ___

Trial 1b

Major divisions ___ X 1.0 = ___
+ # Minor divisions ___ X 0.2 = ___
Salinity in °/oo = ___

Average Salinity for Surface Water

Trial 1a + Trial 1b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___°/oo

SAMPLE #2 (1 Meter Water) (Sample #2)

Trial 2a

Major divisions ___ X 1.0 = ___
+ # Minor divisions ___ X 0.2 = ___
Salinity in °/oo = ___

Trial 2b

Major divisions ___ X 1.0 = ___
+ # Minor divisions ___ X 0.2 = ___
Salinity in °/oo = ___

Average Salinity for 1 Meter Water

La Motte Titration Method
for Salinity

Continued

Trial 2a + Trial 2b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___°/oo

SAMPLE #3 (Bottom Water) (Sample #3)

Trial 3a

Major divisions ___ X 1.0 = ___
+ # Minor divisions ___ X 0.2 = ___
Salinity in °/oo = ___

Trial 3b

Major divisions ___ X 1.0 = ___
+ # Minor divisions ___ X 0.2 = ___
Salinity in °/oo = ___

Average Salinity for Bottom Water

Trial 3a + Trial 3b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___°/oo

Investigator _____
School _____
Instructor _____

M E R
FIELD DATA SHEET

Date _____ Station _____

Turbidity
(Secchi Disk)

DATA:

Trial No.	Distance in Meters that Disk Disappears	Distance in Meters that Disk Reappears	Mean Value

___No. of Trials Total of Means Values____

Total of Mean Values / Number of Trials = ___Avg Dist

1.7 / Average Distance in M = ___Turb Factor

Investigator _____
School _____
Instructor _____

M E R
FIELD DATA SHEET

Date _____ Station _____

Photometric Technique For
Measuring Turbidity (Delta)

DATA

SAMPLE #1 (Surface Water) (Sample #1)

Trial 1a

Meter Reading = ____
Value from the Graph = ___ppm SiO_2

Trial 1b

Meter Reading = ____
Value from the Graph = ___ppm SiO_2

Trial 1a + Trial 1b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm as SiO_2

SAMPLE #2 (1 Meter Water) (Sample #2)

Trial 2a

Meter Reading = ____
Value from the Graph = ___ppm SiO_2

Trial 2b

Meter Reading = ____
Value from the Graph = ___ppm SiO_2

Trial 2a + Trial 2b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm as SiO_2

Photometric Technique for
Measuring Turbidity (Delta)

Continued

SAMPLE #3 (Bottom Water) (Sample #3)

Trial 3a

Meter Reading = ____
Value from the Graph = ___ppm SiO_2

Trial 3b

Meter Reading = ____
Value from the Graph = ___ppm SiO_2

Trial 3a + Trial 3b ÷ 2 =

___ppm + ___ppm ÷ 2 = ___ppm as SiO_2

Investigator _____
School _____
Instructor _____

M E R
FIELD DATA SHEET

Date _____ Station _____

Millipore Technique for
Measuring Turbidity

DATA

SAMPLE #1 (Surface Water)

(Sample #1)

$\frac{145}{\text{(10 grid sq.)}}$ x ___No. of Particles = $\frac{\text{Part}}{250\text{ml}}$

4 x ___Particles/250ml = Particles per liter

(If dilution factor is other than 4, substitute the approp. value.)

SAMPLE #2 (1 Meter Water)

(Sample #2)

$\frac{145}{\text{(10 grid sq.)}}$ x ___No. of Particles = $\frac{\text{Part}}{250\text{ml}}$

4 x ___Particles/250ml = Particles per liter

(If dilution factor is other than 4, substitute the approp. value.)

SAMPLE #3 (Bottom Water)

(Sample #3)

$\frac{145}{\text{(10 grid sq.)}}$ x ___No. of Particles = $\frac{\text{Part}}{250\text{ml}}$

4 x ___Particles/250ml = Particles per liter

(If dilution factor is other than 4, substitute the approp. value.)

Investigator _____
School _____
Instructor _____

M E R
FIELD DATA SHEET

Date _____ Station _____

Nitrate and Nitrite Nitrogen Determination
Colorimetric Method (Delta)

DATA:

SAMPLE No.	Meter Reading	Graph Value	Average Value	
Nitrate/Nitrite-Nitrogen				
SURFACE				
	1a		XXXXXX	
	1b		XXXXXX	___ppm
1 METER				
	2a		XXXXXX	
	2b		XXXXXX	___ppm
BOTTOM				
	3a		XXXXXX	
	3b		XXXXXX	___ppm
Nitrite-Nitrogen				
SURFACE				
	1a		XXXXXX	
	1b		XXXXXX	___ppm
1 METER				
	2a		XXXXXX	
	2b		XXXXXX	___ppm
BOTTOM				
	3a		XXXXXX	
	3b		XXXXXX	___ppm

Nitrate-Nitrogen Determination

Continued

Nitrate-Nitrogen Determination

	Nitrate/Nitrite-N	Nitrite Nitrogen	Nitrate-Nitrogen
SURFACE			___ppm
1 METER			___ppm
BOTTOM			___ppm

Investigator _____
School _____
Instructor _____

M E R
FIELD DATA SHEET

Date _____ Station _____

Phosphate Determination
Colorimetric Method (Delta)

DATA:

SAMPLE No.	Meter Reading	Graph Value	Average Value
Orthophosphate Test			
SURFACE			
1a			XXXXXX
1b			XXXXXX
			____ppm
1 METER			
2a			XXXXXX
2b			XXXXXX
			____ppm
BOTTOM			
3a			XXXXXX
3b			XXXXXX
			____ppm
Total Phosphate Test			
SURFACE			
1a			XXXXXX
1b			XXXXXX
			____ppm
1 METER			
2a			XXXXXX
2b			XXXXXX
			____ppm
BOTTOM			
3a			XXXXXX
3b			XXXXXX
			____ppm

Phosphate Determination

Continued

Polyphosphate Determination

	Total Phosphate	— Ortho-Phosphate =	Poly-phosphate in ppm
SURFACE			____ppm
1 METER			____ppm
BOTTOM			____ppm

Investigator _____

School _____

Instructor _____

APPENDIX II

References

Many portions of the techniques encorporated in this *HANDBOOK OF TECHNIQUES* have been adapted from guides prepared by various companies designed for their products. You may be interested in procuring the original guides, which in many cases contain additional information and techniques which you may wish to adapt as part of the Monitoring Program.

The following list is for your convenience:

DIRECTIONS FOR MODEL 85 OXYGEN METER (Bulletin 85AP) and,
PROCEDURE MANUAL - Delta Scientific Model 50 Portable Laboratory (Bull. 50 AP)
Delta Scientific
120 E. Hoffman Avenue
Lindenhurst, New York 11757

FWPCA METHODS FOR CHEMICAL ANALYSIS OF WATER AND WASTES
November, 1969
U.S. Department of the Interior

INSTRUCTION MANUAL FOR LA MOTTE WATER TEST KIT
Educational Products Division
La Motte Chemical Products Company
Chestertown, Maryland 21620

MILLIPORE EXPERIMENTS IN MICROBIOLOGY
Millipore Corporation
Bedford, Massachusetts 01730

STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, 12th Edition, 1965
American Public Health Association, American Water Works Association, and the Water Pollution Control Federation
Pub. Office: American Public Health Association, Inc.
1740 Broadway, New York, N.Y.

WATER AND WASTEWATER ANALYSIS PROCEDURES - Catalog Number 10
Hach Chemical Company
P.O. Box 907
Ames, Iowa 50010

ERRATA AND ADDENDUM SHEET

AUG 2 1 1973

HANDBOOK OF TECHNIQUES AND GUIDES FOR THE STUDY
OF THE SAN FRANCISCO BAY - DELTA - ESTUARY COMPLEX

MONITORING TECHNIQUES
for the measurement of
PHYSICO-CHEMICAL AND BIOLOGICAL PARAMETERS

PART I

page 17 - OXYGEN DETERMINATION - WINKLER METHOD

The procedure was originally designed to prevent interference from excess iron presumed to be present in local waters. Since this is not the case, the procedure should be modified as follows:

Omit steps 2b through 4 and related chemicals and solutions.

Correction Step 5b. 2.0 ml of hydroxide sodium iodide solution should be added, not 3.0 ml.

page 38 - DETERMINING THE CHLORIDE ION CONCENTRATION AND SALINITY OF WATER - MOHR METHOD

Correction the volume of water sample should be changed from 10 to 5 ml.

Correction - Add - Step 1b If you are working in waters in which the salinity is approximately 5 ppt or greater, add approximately 10 ml of distilled or deionized water. (This will permit the investigator to see the color change more clearly).

Step 1c Add 8 drops of Potassium Chromate Solution (this is the indicator).

Clarification - Step 2 A pinch is equal to approximately the volume of a small pea.

The above method was designed for waters with salinities between 8 to 25 ppt salinity. If you are working in more saline waters, you will have to use a burette of greater than 25 ml capacity.

When determining salinities of waters of less than 8 ppt salinity, it is necessary to make some modifications so that a reasonable volume of $AgNO_3$ is used. You may either increase the volume of the sample OR decrease the normality of the $AgNO_3$. In waters of low salinity, it is not necessary to add distilled water as indicated in step 1b.

In fresh waters, it is customary to indicate salt concentration in ppm.

page 40 - CHLORIDE DETERMINATION TITRAMETRIC TEST (DELTA)

The $AgNO_3$ solution in this test is of low normality designed for waters of low salinity and is not satisfactory in saline waters.

page 48 - MILLIPORE TECHNIQUE FOR MEASURING TURBIDITY

Since many of the waters are very turbid, it will frequently be necessary to use sample volumes as low as 5 ml. When using such small volumes, it is advisable to add approximately 50 ml of distilled or deionized water to the receiving flask of the millipore apparatus and swirl the mixture to insure even distribution of the particles before pulling the solution through the filter.

page 51 - NITRATE AND NITRATE NITROGEN DETERMINATION COLORIMETRIC METHOD (DELTA)
and 55 PHOSPHATE DETERMINATION COLORIMETRIC METHOD (DELTA)

In both of these techniques, it is imperative that the T-50 test cells be scrupulously clean to avoid interferences. Do not wash them with soap or detergent or traces of phosphates may remain and yield erroneous results. Fingerprints on the test cells will cause errors. After using, be sure to rinse them thoroughly in distilled water.

It is suggested that you label the test cells and caps used for these tests so that they do not become contaminated.

page 60 - POPULATION STUDY MUD FLAT OR SANDY BEACH

When laying out the sampling station, attempt to identify the middle of each of the 4 zones identified by Rickets and Calvin instead of sampling at decreasing elevations of 2 feet. This will result in minor changes in the method but should not pose many difficulties. The remaining population studies in this Handbook are based on identification of sampling areas on the basis of these 4 zones.

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The HANDBOOK OF TECHNIQUES AND GUIDES FOR THE STUDY OF THE SAN FRANCISCO BAY-DELTA-ESTUARY COMPLEX was developed and prepared by the staff of the Contra Costa County Department of Education and teachers in Contra Costa and Alameda counties. It is reproduced through the facilities of the Alameda County Superintendent of Schools Office.

Prepared as a course of study for grades 9-14 in aquatic ecology.

For information concerning Project MER or this publication, write to:

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Pleasant Hill, California 94523

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COVER

Crago franciscorum, the Bay Shrimp, was once the most prevalent shrimp in San Francisco Bay. Today, as the result of changes in Bay waters, it is no longer commercially important.

The line drawing of *Crago* sp. was prepared by Margaret Lynn Kiri, student of Ed Springer, at Kennedy High School.

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